University of Alberta

MECHANISMS MEDIATING NUTRITIONAL EFFECTS ON EMBRYONIC SURVIVAL IN CYCLIC GILTS AND WEANED SOWS

by

JIUDE MAO

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Animal Science

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Fall 2000

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-59629-X

Canada

University of Alberta

Library Release Form

Name of Author: **JIUDE MAO**

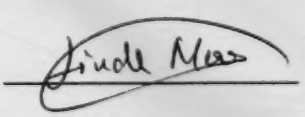
Title of Thesis: **MECHANISMS MEDIATING NUTRITIONAL EFFECTS ON EMBRYONIC SURVIVAL IN CYCLIC GILTS AND WEANED SOWS**

Degree: **Doctor of Philosophy**

Year this Degree Granted: **2000**

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.



204-10533-83 Ave
Edmonton, AB
T6E 2E1

Date: 06/26/00

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faulty of Graduate Studies and Research for acceptance, a thesis entitled

MECHANISMS MEDIATING NUTRITIONAL EFFECTS ON EMBRYONIC SURVIVAL IN CYCLIC GILTS AND WEANED SOWS

Submitted by

JIUDE MAO

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY in ANIMAL SCIENCE

Dr. George Foxcroft (Supervisor)

Dr. Walter Dixon

Dr. Frank Robinson

Dr. Steve Harvey

Dr. Laurie Connor

Date: 23/6/00

ABSTRACT

Suboptimal luteal function is a suggested cause of infertility in pigs. Based on previous work in gilts, an experiment was designed to determine if supplementary progesterone would improve embryonic survival in weaned primiparous sows. Sows were fed ad libitum from farrowing to d 21 of lactation, and then feed restricted from d 22 to weaning at d 28. After weaning, sows were checked for estrus and bred twice. From 36 to 96 after onset of estrus, animals were treated with progesterone or ethyl oleate i.m. every 12 h. On d 28 ± 3 of pregnancy, sows were killed to measure embryonic survival. Progesterone supplementation decreased embryonic survival. Therefore, it seems unlikely that progesterone supplementation could be used to improve embryonic survival in the weaned sow.

The importance of LH in regulating embryonic survival rate was investigated in the second experiment. Primiparous lactating sows were fed to appetite throughout lactation, or fed to appetite during the first three weeks, then feed restricted during the last week of lactation, with, or without concomitant GnRH treatment. After weaning, sows were checked for estrus, bred twice, and slaughtered at d 28 ± 3 of pregnancy. Ovulation rate and embryonic survival were not different among the treatments. Therefore, GnRH treatment in lactation appears to be ineffective for improving reproductive performance after weaning.

The third experiment studied the possibility that effects of the timing of feed restriction and insulin treatment on luteal function in cyclic gilts mediate effects on subsequent fertility. Time of feed restriction did not affect progesterone production or

release, or mRNA expression for steroidogenic enzymes. However, restriction during the second but not the first week of the cycle affected luteal function by decreasing sensitivity to LH stimulation. Insulin treatment restored responsiveness to LH, increased progesterone production and release, and up-regulated steroidogenic enzyme mRNA expression.

In conclusion, neither progesterone nor GnRH treatment increased embryonic survival in sows. However, the lactating sow may be a complex model for studying the mechanisms mediating nutritional effects on reproduction. Insulin treatment in gilts enhanced luteal function and may be a key regulator of embryonic survival.

ACKNOWLEDGMENTS

I am extremely appreciative of the continual encouragement, guidance and assistance for the duration of these studies by my supervisor, Dr. George Foxcroft. I would also like to thank the members of my supervisory committee, Drs. Frank Robinson and Walter Dixon for their input and support. I would like to extend my appreciation to the members of the University of Alberta Swine and Metabolic units for their help during the experiments, and to Shirley Shostak and Rose O'Donoghue for their technical support in the RIA and in vitro laboratories. I wish to thank Drs. Brian Treacy and Denis Balcerzak for the technical assistance in Northern Blotting analysis, RT-PCR and zymography. I would also like to thank the Natural Sciences and Engineering Research Council of Canada, and the Alberta Pork Producers Development Corporation for supplying grants to support these studies.

Without a team effort many aspects of the data reported in this thesis could not have been addressed and in particular the endocrine data obtained from numerous 12 and 24 hour bleeding windows would have surely been sacrificed. The key players involved in this aspect of my experiments were Susan Novak, Fernanda Almeida, Louisa Zak, Shirley Shostak George Foxcroft and John Cosgrove who were always willing to help even on the coldest night.

I express my appreciation to my wife, Xiuping Wei, my daughter, Lisa Weimin Mao, and my parents for their consistent love, support and encouragement.

Dedicated to my wife, Xiuping Wei, and daughter, Lisa Weimin Mao

TABLE OF CONTENTS

Chapter 1.	**Introduction**	1
	Literature cited	5
Chapter 2.	**Literature review**	12
2.1.	Introduction	12
2.2	Early embryonic development and embryonic loss	13
	2.2.1. Early conceptus development	13
	2.2.2. Maternal recognition of pregnancy	15
	2.2.3. Asynchrony between the uterus and embryo	15
	2.2.4. Within-litter embryo diversity	17
	2.2.5. Within-litter diversity: Follicular heterogeneity	18
2.3.	Follicular development and maturation	20
	2.3.1. Gonadotropins sustain follicular growth and steroidogenesis	20
	2.3.2. Follicle recruitment	22
	2.3.3. Follicle selection	22
	2.3.4. Local regulation of folliculogenesis	23
	2.3.4.1. Inhibin-related peptides	23
	2.3.4.2. Insulin-like growth factor-I system	25
	2.3.4.3. Insulin	28
	2.3.5. Follicle development during lactation and after weaning	29
2.4.	Luteal function and its regulation	30
	2.4.1. Luteinization and associated changes	30
	2.4.2. Luteal cell types	31

2.4.3. Interactions among luteal cells 32

2.4.3.1. Large- and small- luteal cell interactions 32

2.4.3.2. Interactions between luteal cells and other cells 34

2.4.4. Progesterone synthesis 35

2.4.5. Progesterone as a mediator of embryo survival 36

2.4.6. Causes of inadequate luteal function 37

2.5. Hormone secretion during lactation and after weaning 38

2.5.1. Neuro-endocrine basis of lactational anestrus 39

2.5.2. Prolactin secretion and the relationship with LH 39

2.5.3. GnRH, FSH and LH secretion and effects of litter size and feed intake 41

2.5.4. Endocrinology of the sow at weaning 43

2.5.5. Weaning-to-estrus interval 44

2.6. Metabolic cues and LH secretion 45

2.6.1. Nutrient availability 46

2.6.2. Insulin 48

2.6.3. Leptin 49

2.6.4. Neuropeptide Y 52

2.6.4.1. Excitatory effects of NPY on LH secretion 53

2.6.4.2. Inhibitory effects of NPY on LH secretion 55

2.6.4.3. Effects of food restriction on NPY and LH secretion 55

2.6.4.4. Regulation of NPY by leptin and insulin 56

2.7. Conclusions 57

Literature cited 59

Chapter 3.	**Progesterone therapy during early pregnancy and embryonic survival in primiparous weaned sows**	
	Introduction	101
	Materials and methods	102
	Results	105
	Discussion	106
	Literature cited	111
Chapter 4.	**Reproductive, metabolic, and endocrine responses to feed restriction and GnRH treatment in primiparous lactating sows**	
	Introduction	122
	Materials and methods	123
	Results	127
	Discussion	131
	Literature cited	137
Chapter 5.	**Luteal function in the immediate post-ovulatory period in feed-restricted and insulin-treated pigs: Progesterone production *in vitro* and mRNA expression for key steroidogenic enzymes**	
	Introduction	152
	Materials and methods	154
	Results	161
	Discussion	163
	Literature cited	170
Chapter 6.	**General discussion**	191
	Literature cited	199

LIST OF TABLES

Table		Page
3.1.	Composition of experimental diets as formulated.	114
3.2.	LSMeans (± SEM) of sow feed intake during and after lactation, sow body weight and body weight change, sow backfat thickness at P2 and backfat thickness change, litter weight and piglet growth rate during lactation in control and progesterone-treated sows.	115
3.3.	LSMeans (± SEM) of plasma progesterone concentrations (ng/ml) at 36, 48, 72, and 96 h after onset of standing heat in the control (n = 14) and progesterone-treated sows (n = 15).	116
3.4.	LSMeans (± SEM) of weaning-to-estrus interval (WEI), ovulation rate, total number of embryos, total embryonic survival rate, number of viable embryos, viable embryonic survival rate and crown-rump length at day 28 ± 3 of pregnancy in control and progesterone-treated sows.	117
4.1.	Composition of experimental diets as formulated.	141
4.2.	LSMeans (± SEM) of feed intake, sow body weight, body weight change, backfat thickness, backfat change, and litter weight at weekly intervals during lactation in AA (n=8), AR (n=12), and AR + GnRH (n=12) sows.	142
4.3.	LSMeans (± SEM) of plasma IGF-I, pre- and post-prandial insulin concentrations and LH pulse frequency (per 12 h period) on d 21, before and after weaning on d 28 of lactation.	143
4.4.	LSMeans (± SEM) of weaning-to-estrus interval, ovulation rate, number of viable embryos, embryo survival rate, and embryo crown-rump length in AA, AR and AR + GnRH sows.	144
4.5.	LSMeans (± SEM) of plasma progesterone concentrations at 36, 48, 72, and 96 h after onset of standing heat in AA, AR, and AR + GnRH sows.	145
5.1.	LSMeans (± SEM) of progesterone (P4) content in pooled luteal tissue (CL) before (time zero progesterone, T0 P4) and after culture, and P4 production and release during culture by luteal tissue, and P4 production by dispersed luteal cells, recovered from HR, HR+I, and RH gilts.	175

LIST OF FIGURES

Figure		Page
1.1.	Estimates of prenatal mortality throughout gestation.	8
1.2.	Composition of litter size at farrowing in gilts and primiparous sows.	10
2.1.	Estrous cycle of the pig.	93
2.2.	Three endocrine phases of lactation and follicle development in the lactating sow.	95
2.3.	Progesterone synthesis pathways within the luteal cell.	97
2.4.	The relationships between insulin, leptin, NPY, pituitary gonadotropins, and ovarian function in anabolic and catabolic states.	99
3.1.	Correlation between the number of corpora lutea and progesterone concentrations at 72 h after onset of standing heat in the control sows (n = 14).	118
3.2.	Relationship between plasma progesterone at 72 h after onset of standing heat and embryonic survival rate in all sows (n = 29).	120
4.1.	Plasma leptin concentrations on d 21 in all sows (n = 29), d 28 of lactation and the day after weaning before feeding in AA (n = 7) and pooled AR and AR + GnRH sows (n = 22)	146
4.2.	Plasma LH maximum, mean, and minimum concentrations and FSH concentrations on d 21 and 28 before and after weaning in AA, AR, and AR + GnRH sows.	148
4.3.	LH profiles representing AA, AR, and AR + GnRH sows on d 28 before, and after, weaning (0 is weaning time).	150
5.1.	Correlation between progesterone (P4) production (A) and release (B) by minced luteal tissue in vitro (ng/mg/3 h) and ovulation-to-surgery time interval in HR, HR+I and RH gilts.	176
5.2.	Progesterone (P4) production (A) and release (B) *in vitro* by luteal tissue from HR, HR+I, and RH gilts, and progesterone production by dispersed luteal cells (C; pooled data) in response to LH stimulation.	178
5.3.	Correlation between progesterone production by minced luteal tissue *in vitro* and oviductal (A), and peripheral (B) progesterone (P4) concentrations at the time of surgery, and between progesterone release by luteal tissue *in vitro* and oviductal (C), and peripheral (D) progesterone concentrations at the time of surgery (n=44).	180

5.4. StAR, P450scc, and 3beta-HSD mRNA expression in luteal tissue from HR, HR+I and RH groups. 183

5.5. StAR protein (A), P450scc (B), and 3beta-HSD (C) mRNA expression in luteal tissue relative to ovulation-to-surgery time interval in HR, HR+I and RH gilts. 185

5.6. Correlation between StAR protein (A), P450scc (B), and 3beta-HSD (C) mRNA expression in luteal tissue and progesterone production by luteal tissue *in vitro* (n=29). 187

5.7. A). mRNA expression for MMP-2, MMP-9, TIMP-1, and TIMP-2 in luteal tissue from HR, HR+I and RH gilts (n=30);
B). Representative TIMP-1 and G3PDH RT-PCR amplicons in luteal tissue from HR, HR+I, and RH gilts. 189

LIST OF ABBREVIATIONS

2-DG	2-deoxyglucose
3β-HSD	3beta-hydroxysteroid dehydrogenase
ARC	arcuate nucleus
cAMP	cyclic adenosine monophosphate
CL	corpus luteum
CNS	central nervous systems
EGF	epidermal growth factor
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin
i.c.v.	intracerebroventricular
IFN-γ	interferon-gamma
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IL-1β	interleukin 1beta
LH	luteinizing hormone
ME	median eminence
MMP	matrix metalloproteinases
MPOA	medial preoptic area
mRNA	messenger ribonucleic acid
NAL	naloxone
NMDA	N-methyl-d, l-aspartate
NPY	neuropeptide Y
OVX	ovariectomy
P450 arom	aromatase cytochrome P450

P450c17	17α-hydroxylase/C17-20 lyase
P450 scc	cholesterol side-chain cleavage cytochrome P450
PGF2α	prostaglandin F2alpha
PGE	prostaglandin E
PRL	prolactin
RT-PCR	reverse transcription-polymerase chain reaction
StAR protein	steroidogenic acute regulatory protein
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinases
VEGF	vascular endothelial growth factor
WEI	weaning-to-estrus interval

GLOSSARY

Ad libitum feeding: a feeding system in which pigs can access feed at any time.

Apoptosis: Cells undergoing programmed cell death often exhibit distinctive morphologic changes, collectively referred to as apoptosis.

Backfat thickness at P2: the fat depth 65 mm from midline at the position of the last rib, which is the universally adopted site for assessment of adiposity tissue in pigs.

Follicle atresia: a process by which follicles undergo regression and are not destined to produce a functional ovum.

Gilt: immature female pig prior to production of her first litter.

Primiparous sow: sow bearing her first litter.

Sow: an adult female pig of any age that has farrowed.

To appetite feeding: a feeding system in which pigs only can access feed for defined periods of time.

Zero weaned sows: sows that are weaned at farrowing.

CHAPTER 1
INTRODUCTION

In the swine industry, productivity and profit are based on the number of live pigs weaned per sow per year. Therefore, sow reproductive efficiency is a prime concern and is controlled by litter size at birth, the number of pigs surviving from birth to weaning and the interval between the birth of successive litters (farrowing interval). Improvements in herd management have resulted in reductions in farrowing interval and pre-weaning mortality (Legault, 1985), but litter size has changed little during the past 80 years (Ashworth and Pickard, 1998). Litter size is dependent on ovulation rate, fertilization rate, and prenatal survival during pregnancy. Ovulation rate, the number of eggs shed by a sow, is the maximum potential litter size. Fertilization rate is generally over 95% (Perry and Rowlands, 1962; Polge, 1982; Lambert et al., 1991; Soede et al., 1992) when females are bred at the optimum time. If mating management is optimized, any observed reduction in litter size below the number of ovulations is, therefore, largely due to prenatal losses. The importance of variation in prenatal mortality as a source of variation in litter size has been reported (Van der Lende et al., 1994). Prenatal mortality is divided into embryonic and fetal mortality. Embryonic mortality generally refers to fertility losses during the embryonic period from fertilization to completion of differentiation, arbitrarily taken as d 30 of gestation. Embryonic survival rate may be the most important factor determining litter size born. After correcting for fertilization failure, about 30 to 40% of fertilized eggs die during the first 30 d of gestation (refer to Figure 1.1) (Pope, 1994; Ashworth and Pickard, 1998), with an additional 10 to 20% of fetuses dying in the remaining period in gilts and primiparous sows (Pope and First, 1985; Ashworth, 1991; Pope, 1994; Ashworth and Pickard, 1998; Figure 1.2). However, data from modern genotypes suggests that the distribution of prenatal loss may change as sows reach higher parities, resulting in 50% or more prenatal loss in the post-implantation period (Foxcroft, 1997).

Embryonic loss is not inevitable, as up to 20% of gilts have no embryonic loss (Dziuk, 1987; Lambert et al., 1991; Jindal et al., 1996, Almeida et al., 2000). However, the mechanisms of embryonic mortality have not been completely understood. In all

likelihood, no one explanation may account for all embryonic loss. Among the many factors suggested to contribute to embryonic loss are environmental, genetic, nutritional and biochemical factors which interact, and which ultimately impinge on the embryo's developmental environment and on the relationships between embryos occupying the same uterus. Asynchrony between the embryo and the uterine environment, and within a litter, has been suggested to be the underlying cause of most embryonic mortality, with other factors modifying the degree of asynchrony (Pope and First, 1985; Dziuk, 1987; Pope et al., 1990; Pope, 1994; Ashworth and Pickard, 1998). Recently, nutritional effects on embryonic survival have received much attention, especially feed restriction. It has been demonstrated several times that feed restriction before ovulation decreases embryonic survival in both cyclic gilts (Ashworth et al., 1995; Almeida et al., 2000) and in primiparous lactating sows (Zak et al., 1997a). Such nutrition-reproduction interactions have become the focus of considerable research in recent years.

Lactating sows have a high requirement for energy and protein, as approximately 75% of their energy requirements go to milk production (Aherne and Kirkwood, 1985; Noblet et al., 1990). However, primiparous sows frequently can not consume sufficient feed during lactation to meet the nutritional requirements for milk production and maintenance (Williams, 1985), and consequently, they are in negative energy balance. The negative energy balance during this period is compensated by an increased mobilization of body reserves, such as lipid and protein, so as to maintain milk production (Mullen and Williams, 1990). Greater embryonic mortality in sows as a result of previous low feed intake during lactation has been reported by King and Williams (1984), Hughes et al. (1984) and Kirkwood et al. (1987a, b; 1990). In the studies by Zak et al. (1997a), different patterns of catabolism in lactation, as a consequence of differences in feeding regimen, affected subsequent reproductive performance of the primiparous sow. In that study sows were either fed to appetite throughout 28-day lactation (AA), fed to appetite from farrowing to d 21 of lactation and then restrict fed to 50% from d 22 to d 28 (AR), or restrict fed from farrowing to d 21 and then fed to appetite from d 22 to d 28 (RA). Weaning-to-estrus interval increased in the feed restricted sows (AR and RA) and ovulation rate was lower compared to AA sows. Interestingly, embryonic survival did not differ between RA and AA sows (86.5% vs

87.5%), but was significantly lower in AR sows (64.4%). This study demonstrated that not only the overall feeding level in sows, but also the pattern of tissue loss, is important for regulating reproductive performance.

Similarly, young cyclic gilts have a high nutritional requirement for growth and feed intake before mating has a great influence on subsequent embryonic survival. Gilts feed-restricted prior to mating not only had a lower ovulation rate but also lower embryo survival than gilts receiving a high diet during the same periods (Ashworth et al., 1995). In an experiment modelled on the work of Zak et al. (1997a), moderate feed restriction (75% of ad libitum feed intake) during the second week of the estrous cycle in gilts did not affect ovulation rate but decreased embryonic survival at d 28 of pregnancy, compared with either the non-restricted gilts or gilts feed restricted during the first week of the cycle (Almeida et al., 2000), again suggesting that the timing of nutritional effects can be critical.

Severe feed restriction suppressed pulsatile LH secretion and affected follicular development in gilts (Britt et al., 1988; Booth et al., 1994, 1996) and primiparous lactating sows (Zak et al., 1997a,b; Quesnel et al., 1998). Reduced embryo survival as a result of reduced feed intake may therefore be related to reduced gonadotropin secretion (Foxcroft et al., 1995; Zak et al., 1997a; Quesnel et al., 1998). Impaired gonadotropin secretion could affect follicular development, and thereafter affect embryonic survival. In order to study the physiological mechanisms mediating nutritional effects on embryonic survival in pigs, it is important to understand the associations between feed restriction, gonadotropin secretion, follicular development, luteal function and embryonic survival. The purpose of the literature review presented in Chapter 2 of this thesis is to provide such an understanding.

The objective of the series of studies described in the later section of the thesis was to further clarify the mechanisms regulating nutritional effects on embryonic survival using the primiparous lactating sow model developed by Zak et al. (1997a) and the cyclic gilt model developed by Almeida et al. (2000). The initial study (Chapter 3) was to determine if negative effects of lactational feed restriction on embryonic survival in the primiparous weaned sow could be abrogated by administration of progesterone in the early pregnancy.

Feed restriction has been shown to depress pulsatile LH secretion in lactating sows and reduce embryo survival rate, independent of ovulation rate (Zak et al., 1997a; Quesnel et al., 1998). Experiment 2 (Chapter 4) addressed the hypothesis that exogenous GnRH treatment in feed-restricted sows, which restored LH secretion to that seen in unrestricted sows in late lactation, would ameliorate effects of restrict feeding on subsequent embryonic survival at d 28 of pregnancy.

Progesterone production in early pregnancy is suggested to be important in mediating embryonic survival in pigs (Hunter et al., 1996; Foxcroft, 1997; Ashworth and Pickard, 1998; Van den Brand et al., 2000). Insulin has been established as one modulator of reproductive function. In the third experiment (Chapter 5), luteal function in previously feed-restricted and insulin-treated gilts was studied in luteal tissue recovered in the immediate post-ovulatory period by determining expression of mRNA encoding key steroidogenic enzymes, and progesterone production and release *in vitro*.

Having presented these experimental results, Chapter 6 of this thesis presents a general discussion on the overall impact of the research on our understanding of embryonic loss in the pig, and further research is proposed.

LITERATURE CITED

Aherne FX, Kirkwood RN. Nutrition and sow prolificacy. J Reprod Fertil Suppl 1985; 33: 169-183.

Almeida FRCL, Kirkwood RN, Aherne FX, Foxcroft GR. Patterns of feed intake during the estrous cycle in gilts and the effects on conceptus development and embryonic survival. J Anim Sci 2000; (in press).

Ashworth CJ. Embryo development. Pig News and Inf 1991; 12: 551-554.

Ashworth CJ, Antipatis C, Beattie L. Effect of pre- and post-mating nutritional status on embryo survival and uterine function in the pig. J Reprod Fertil Abstr Ser 1995; 16: 27 (Abstract).

Ashworth CJ, Pickard AR. Embryo survival and prolificacy. In: Progress in pig science. Nottingham University Press. 1998; 303-325.

Booth PJ, Cosgrove J, Foxcroft GR. Endocrine and metabolic responses to realimentation in feed-restricted prepubertal gilt: associations among gonadotrophins, metabolic hormones, glucose, and utero-ovarian development. J Anim Sci 1996; 74: 840-848.

Booth PJ, Craigon J, Foxcroft GR. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J Anim Sci 1994; 72: 2415-2424

Britt JH, Armstrong J, Cox N. Metabolic interfaces between nutrition and reproduction in pigs. 11th Int Conf Anim Reprod AI. Plenary and Symposia. 1988; 5: 117-125.

Dziuk PJ. Embryonic loss in the pig: an enigma. Manipulating Pig Production. Proceeding Inaugural Conference, Australia, Pig Science Association. 1987; 28-39.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Foxcroft GR, Aherne FX, Clowes EJ, Miller HM, Zak LJ. Sow fertility: the role of suckling inhibition and metabolic status. In: Ivan M (ed), Animal Science Research and Development: Moving towards a new century. 1995; Ottawa, Canada. 377-391.

Hughes PE, Henry RW, Pickard DW. The effects of lactation food level on subsequent ovulation rate and embryonic survival in the sow. Anim Prod Sci 1984; 67: 943-950.

Hunter MG, Picton HM, Biggs C, Mann GE, McNeilly AS, Foxcroft GR. Periovulatory endocrinology in high ovulating Meishan sows. J Endocrinol 1996; 150: 141-147.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. J Anim Sci 1996; 74: 620-624.

King RH, Williams IH. The effect of nutrition on the reproductive performance of first-litter sows. 1. Feeding level during lactation and between weaning and remating. Anim Prod 1984; 38: 241-247.

Kirkwood RN, Baidoo SK, Aherne FX, Sather AP. The influence of feeding level during lactation on the occurrence and endocrinology of the postweaning estrus in sows. Can J Anim Sci 67: 1987a; 405-415.

Kirkwood RN, Lythgoe ES, Aherne FX. Effect of lactation feed intake and gonadotrophin-releasing hormone on the reproductive performance of sows. Can J Anim Sci 1987b; 67: 715-719.

Kirkwood RN, Baidoo SK, Aherne FX. The influence of feeding level during lactation and gestation on the reproductive performance of second parity sows. Can J Anim Sci 1990; 70: 1119-1126.

Lambert E, Williams DH, Lynch PB, Hanrahan TJ, McGeady TA, Austin FH, Boland MP, Roche JF. The extent and timing of prenatal loss in gilts. Theriogenology 1991; 36: 655-665.

Legault C. Selection of breeds, strains and individual pigs for prolificacy. J Reprod Fertil Suppl 1985; 33:151-166.

Mao J, Foxcroft GR. Progesterone therapy during early pregnancy and embryonal survival in primiparous weaned sows. J Anim Sci 1998; 76: 1922-1928.

Mao J, Zak LJ, Cosgrove JR, Shostak S, Foxcroft GR. Reproductive, metabolic, and endocrine responses to feed restriction and GnRH treatment in primiparous, lactating sows. J Anim Sci 1999; 77: 724-735.

Mullan BP, Williams IH. The chemical composition of sows during their first lactation. Anim Prod 1990; 51: 375-387.

Noblet J, Dourmad JY, Etienne M. Energy utilization in pregnant and lactating sows: modeling of energy requirements. J Anim Sci 1990; 68: 562-572.

Perry JS, Rowlands IW. Early pregnancy in the pig. J Reprod Fertil 1962; 4: 175-188.

Polge C. Embryo transplantation and presevation. In: Cole OJ, Foxcroft GR (eds.), Control of Pig Reproduction. Butterworth Scientific, London. 1982; 277-291.

Pope WF.. Embryonic Mortality in Swine. In: Zavy MT, Geisert RD (eds.). Embryonic Mortality in Domestic Species. CRC Press. 1994; 53-77.

Pope WF, First NL. Factors affecting the survival of pig embryos. Theriogenology 1985; 23: 91-105.

Pope WF, Xie S, Broermann DM, Nephew KP. Causes and consequences of early embryonic diversity in the pig. J Reprod Fertil Suppl 1990; 40: 251-260.

Quesnel H, Pasquier A, Mounier AM, Prunier A. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J Anim Sci 1998; 76: 856-863.

Soede NM, Noordhuizen JPTM, Kemp B. The duration of ovulation in pig, studied by transrectal ultrasonograph, is not related to early embryonic diversity. Theriogenology 1992; 38: 653-666.

Van den Brand H, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: II. Effects on periestrus hormone profiles and embryonal survival. J Anim Sci 2000; 78: 405-411.

Van der Lende T, Soede NM, Kemp BV. Embryo mortality and prolificacy in the pig. In: Cole DA, Wiseman J, Varley MA (eds.), Principles of Pig Science. Nottingham: Nottingham University Press, UK. 1994; 297-317.

Williams IH. Feed intake and reproduction in pigs. Proc Nutr Soc Aust 1985; 10: 63-69.

Zak LJ, Cosgrove JR, Aherne FX, Foxcroft GR. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J Anim Sci 1997a; 75: 208-216.

Zak LJ, Xu X, Hardin RT, Foxcroft GR. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J Reprod Fertil 1997b; 110: 99-106.

Figure 1.1 Estimates of prenatal mortality throughout gestation (Redrawn after Ashworth and Pickard, 1998).

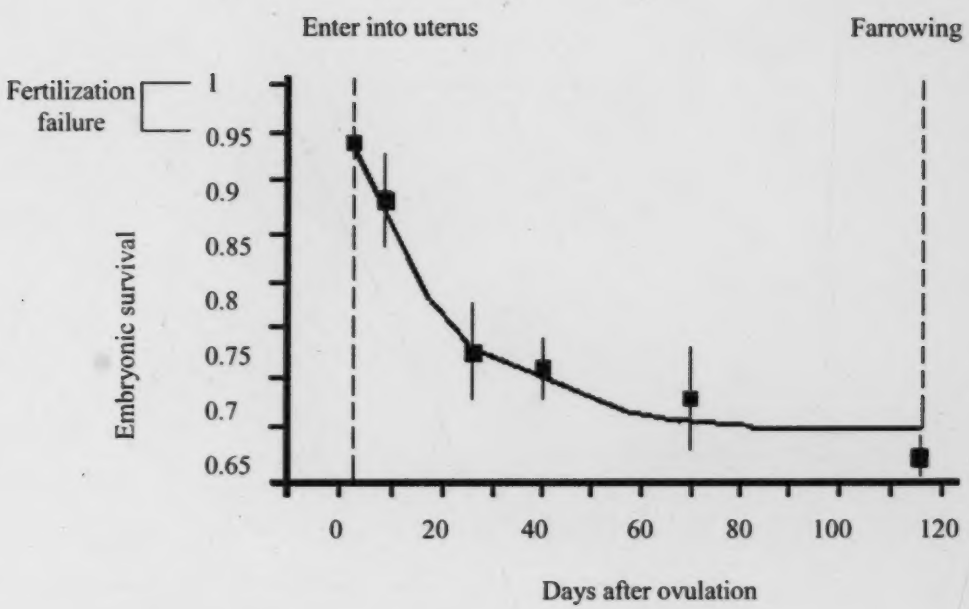
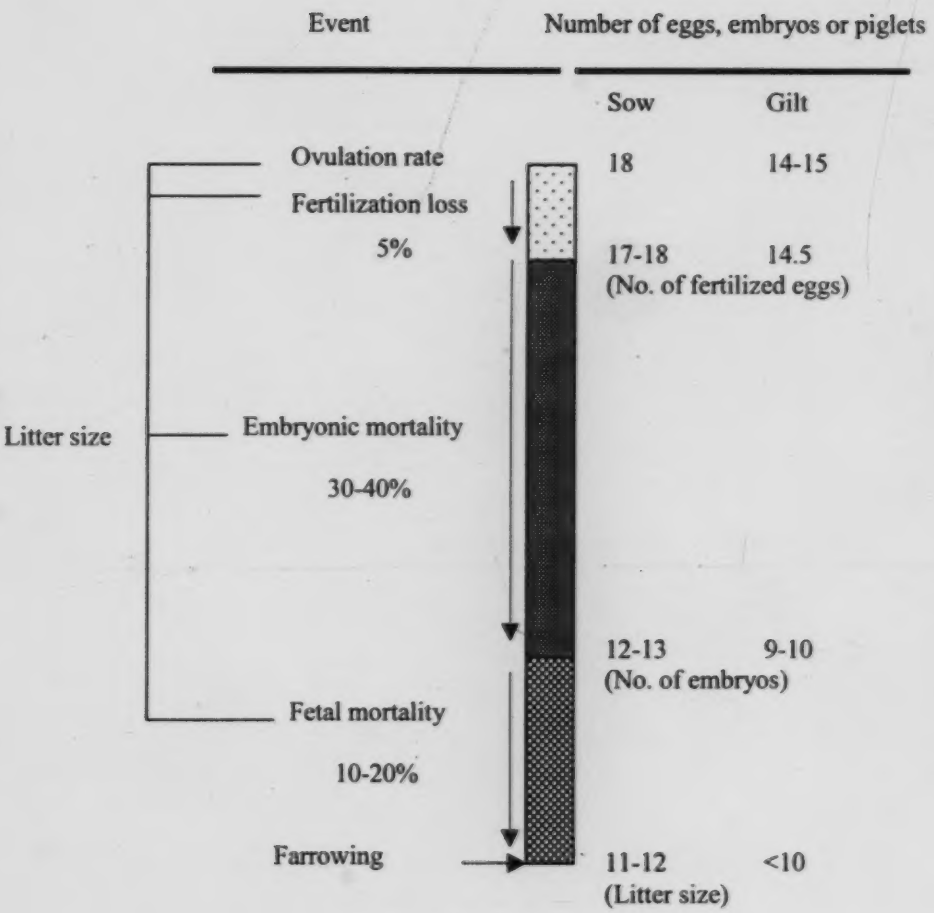

Enter into uterus
Farrowing
Fertilization failure
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
Embryonic survival
0
20
40
60
80
100
120
Days after ovulation

Figure 1.2. Composition of litter size at farrowing in gilts and primiparous sows (based on the data of Soede et al., 1992; Zak et al., 1997a; van den Brand 2000 and reviews of Pope and First, 1985; Lambert et al., 1991; Ashworth, 1991; Pope, 1994; Ashworth and Pickard, 1998).


Event
Number of eggs, embryos or piglets
Sow
Gilt
Ovulation rate
18
14-15
Fertilization loss
5%
17-18
14.5
(No. of fertilized eggs)
Litter size
Embryonic mortality
30-40%
12-13
9-10
(No. of embryos)
Fetal mortality
10-20%
Farrowing
11-12
<10
(Litter size)

CHAPTER 2
LITERATURE REVIEW

2.1. Introduction

The mechanisms mediating nutritional effects on embryonic survival in pigs have not been clarified (Ashworth, 1994; Foxcroft, 1997). Nutritional status has great effects on LH secretion, but both central and local mechanisms mediate nutritional effects on reproductive performance (I'Anson et al., 1991; Cosgrove and Foxcroft, 1996; Foxcroft, 1997). The objectives of this literature review are to provide an updated understanding of the associations between feed restriction, gonadotropin secretion, follicular development, luteal function and embryonic survival in the pig, and to describe the mechanisms involved. The first section of the literature review covers the development of the porcine embryo from fertilization, through implantation and embryonic development to d 30 of pregnancy, including the physical and biochemical development of the embryo. Around d 11 of pregnancy, embryos acquire the capacity to synthesize estradiol. Estradiol is the signal for maternal recognition of pregnancy and may modify the microenvironment of embryo development to favor its own development, but may be harmful to adjacent littermates. Such asynchrony within litter, and between embryo and uterus, is believed the primary cause of embryo loss and may result from follicular heterogeneity (Pope et al., 1990; Pope, 1994). Therefore, the physiology of follicular development, including follicle recruitment and selection, will be reviewed in the second section. Gonadotropins are the major regulators of the later stages of follicular development, but non-steroid local factors such as insulin-like growth factor-I and inhibin-related peptides are also important and will be addressed in this section. After ovulation, granulosa cells and theca cells continue to develop into the corpus luteum. Optimal luteal function in the early pregnancy stage is essential for oviductal and uterine secretion and suboptimal luteal function is associated with lower embryonic survival. Luteal function and its regulation, and causes for sub-normal luteal function will therefore be covered in section 3. Gonadotropins stimulate follicular development during the follicular phase. The lactating and weaned sows were used to investigate the regulation of embryonic survival, which were reported in later Chapters. Therefore, the secretion of gonadotropins during

lactation and after weaning will be reviewed in section 4. As discussed above, severe feed restriction depresses gonadotropin secretion and the possible mechanisms mediating nutritional effects on gonadotropin secretion are also reviewed in the final section.

In the literature review presented, the pig will be used as the main species for discussion; relevant literature from other species is quoted as indicated to present a more comprehensive picture.

2.2. Early embryonic development and embryonic loss

2.2.1. Early conceptus development

The pig is a polytocous species. During each estrous cycle, several follicles develop simultaneously and ovulate. The duration of spontaneous ovulation lasts 1 to 3 h (Soede et al., 1992). If a pig is mated at least 6 h before ovulation, the first oocyte released at ovulation is the first fertilized, and the last oocyte ovulated is the last fertilized (Xie et al., 1990b). Fertilization takes place in the ampulla of the oviduct, near the ampullary-isthmic junction (Hunter, 1977). Within a few hours after fertilization, the fertilized eggs are moved to the ampullary-isthmic junction and remain there for about 36 h. Embryos enter the uterus from the oviducts 48 h after ovulation, on d 3.5 to 4 of gestation at the 4- to 8-cell stage (Oxenreider and Day, 1965; Broermann et al., 1990). The rate of passage through the oviduct may be greatly speeded by administration of exogenous progesterone to the female before ovulation (Day and Polge, 1968). By day 5, inner and outer cells of 12- to 16- cell morulae can be differentiated (Stroband and Van der Lende, 1990). It is believed that the inner cells will differentiate to form the inner cell mass, while the outer cells differentiate to trophectoderm. On days 6 to 7 of gestation, the embryos, which are in the early blastocyst stage and comprise 16-32 cells, hatch from the zone pellucida (Hunter, 1977).

Between d 7 and 12 after fertilization, the embryos migrate through the uterine horns and subsequently redistribute themselves over the full length of both horns (Dhindsa et al., 1967; Dziuk, 1985). This spacing process is often accompanied by trans-uterine migration, even if the number of ovulations between the ovaries has been equal (Dziuk et al., 1964). Both estradiol-17β from the embryo and histamine from the embryo and uterus are involved in intrauterine migration of embryos (Pope et al., 1982a). As a

result of migration, the embryos will be equally spaced from each other. Before d 25 to 30, uterine space has little influence on embryo survival (Pope and Day, 1972; Dziuk, 1985). However, Morgan et al. (1987) and Gries et al. (1989) have shown that embryos subjected to an estrogen-altered uterine environment on d 9 and 10 elongate normally but do not attach. Instead, they degenerate and die by d 16 to 18 of gestation. From this observation, Pusateri et al. (1990) suggested that the less developed embryos adversely affected by high levels of estradiol produced by their more advanced littermates do not space normally and fail to attach.

By d 11 or 12 of gestation, the embryos are spherical and their diameter increases during this period up to 10 mm (Stroband et al., 1984). Subsequently the embryos start to elongate rapidly from the 9-10 mm spherical stage to the 1 m long filamentous form by d 16 (Anderson, 1978). This elongation is initially mainly due to cell reorganization and not to cell division (Geisert et al., 1982b; Pusateri et al., 1990); however, continued elongation and growth of the conceptus does involve an increase in cell number. Almost simultaneously with the onset of elongation, the embryos develop aromatase activity (Van der Meulen et al., 1989) and start to synthesize and secrete estrogens (Heap et al., 1979; Bazer et al., 1982; Pusateri et al., 1990). These estrogens induce (1) maternal recognition of pregnancy, through maintenance of the corpora lutea and thus the continuation of progesterone secretion (Bazer and Thatcher, 1977; Flint et al., 1983); (2) embryonic migration (Pope et al., 1982a); (3) elongation of the uterus (Pope and First, 1985); and (4) stimulation of uterine secretion from the endometrium (Geisert et al., 1982a; Pope, 1988). These changes in embryonic and uterine development during the early stage of gestation are highly interrelated and interdependent (Geisert et al., 1990). Alterations in these sequential events can affect the viability of an embryo and occur naturally. The embryo-mediated prolongation of the lifespan of the corpora lutea is called maternal recognition of pregnancy.

Around d 13 to 14 attachment commences, with a loose contact between trophoblast and uterine membranes, and is completed by interdigitation of uterine and trophoblastic microvilli after d 18 (Dantzer, 1985). Each blastocyst occupies only a relatively short length of the uterus (Perry and Rowlands, 1962). Concomitant with this, the trophoblast and embryoblast differentiate to form the extraembryonic membranes of

chorion, the amnion, yolk sac and allantois (placenta) and specialised structures of the conceptus, respectively (Ashworth, 1991b).

Several dramatic changes occur between d 18 and d 30 of gestation. The length of the uterine horn increases by about 40% (Wu et al., 1988); there is a great increase in uterine blood flow and the volume of fluid in the allantoic sac increases markedly from about 4 ml on d 18 to a peak of approximately 190 ml on d 30 of gestation (Goldstein et al., 1980).

The studies by Scofield et al. (1974) and several reviews (Pope and First, 1985; Van der Lende and Schoenmaker, 1990; Pope, 1994; Van der Lende et al., 1994; Ashworth and Pickard, 1998) indicate that the major part of embryo mortality occurs before d 18 of pregnancy; of which about one third occurs before d 9 and about two thirds between d 9 and 18. A high proportion of embryonic mortality occurs around the time of maternal recognition of pregnancy and implantation.

2.2.2. Maternal recognition of pregnancy

The uterine environment can support the development of a similarly aged blastocyst until d 9 of pregnancy (Polge, 1982). After this stage the embryo itself will modify the uterine environment through a cascade of events, a process that is called maternal recognition of pregnancy. Maternal recognition of pregnancy is defined as the method by which the conceptus prolongs the functional lifespan of the corpora lutea established after ovulation (Roberts et al., 1993). The embryo signals its presence to the dam by producing a pulse of estradiol at d 11 and a second and more prolonged phase of estradiol secretion between d 14 and 16 of pregnancy (Geisert et al., 1987). In swine, the rescue of the corpus luteum which prevents luteolysis and allows continued synthesis of progesterone, is achieved by embryonic estradiol redirecting PGF2α secretion towards the uterine lumen (Gross et al., 1988) and presents an effective luteolytic signal from interfering with ovarian progesterone synthesis (Bazer et al., 1984).

2.2.3. Asynchrony between the uterus and embryo

In swine, over 30 % of the eggs ovulated fail to materialize as live born piglets. Embryonic mortality reduces sow productivity in the swine industry. Although the causes of embryonic mortality are still poorly understood, embryos carried by individual

mothers vary considerably in their stage of development. For example, on day 11 (Day 0 = estrus) littermate embryos can be in the spherical, ovoidal, tubular and filamentous stages of development. Therefore, it has been proposed that the resulting asynchronous development between the uterus and a proportion of embryos is one cause of embryonic loss in swine (Dziuk, 1987; Pope, 1994).

For successful embryo transfer, it is essential that the embryo and uterine environment be physiologically synchronized (Pope et al., 1982b). As reviewed by Polge (1982), asynchronous embryo transfer techniques demonstrated that the success of establishing pregnancy with the transfer of advanced (+24 to +48 h) embryos was comparable to that obtained using embryos synchronous with the recipients' estrous cycle. But as found by Jarrell et al. (1990) and Geisert et al. (1991), failure to establish pregnancy with embryos transferred 48 h behind the recipients estrous cycle is not related to trophoblastic elongation or synthesis of estrogen. The early loss is caused by rapid deterioration of the embryos within 24 h following transfer. These data indicate that early embryonic survival is not inhibited when embryos are advanced compared with the recipient's uterine environment, but younger embryos are intolerant of an advanced uterine environment.

Treatment with estradiol-17beta on d 11 stimulates a physiological response in uterine release of calcium, protein and uteroferrin as seen during early pregnancy (Geisert et al., 1982a). As conceptus elongation is not affected by estrogen treatment (Geisert et al., 1991), embryonic mortality could result from either inappropriate early timing of uterine secretion (Morgan et al., 1987), or the inability of the conceptus to attach to the uterine epithelial glycocalyx (Gries et al., 1989). Uterine epithelial glycocalyx synthesis is stimulated by embryonic estrogen on d 11 to 12 of pregnancy (Blair et al., 1991) or after administration of estradiol-17beta (Key and King, 1988). Aplin (1997) accumulated a large body of evidence to suggest that attachment is controlled by the steroidal induction of one or more adhesion molecules at the luminal epithelial cell surface, converting the epithelium from a non-receptive to a receptive state. Therefore, the less developed embryos may not induce this epithelial conversion in time and are thus unable to attach to the uterine surface because of the short receptive phase for embryo implantation (Pusateri et al., 1990).

2.2.4. Within-litter embryo diversity

It has also been proposed that embryonic loss may result from asynchronous development among littermates, a phenomenon that leads to poorly coordinated biochemical interactions between the conceptuses and the maternal systems (Pope, 1988; Pope et al., 1990; Roberts et al., 1993; Ashworth and Pickard, 1998). There is a great variance in morphology, and in protein and DNA content of embryos among littermates at the same stage of pregnancy (Pusateri et al., 1990). Usually, smaller, less advanced conceptuses are presumed to be lost because they become out of phase with the uterine environment and Pope et al. (1982b) proved that the older, more developed, embryos had a preferential chance of survival. However, the death of less advanced embryos is not apparently due to them being less viable than the embryos that are more advanced. Wilde et al. (1988) segregated d 7 blastocysts into three size groups, the largest, intermediate and smallest, and transferred the smallest and the largest embryos into individual ligated horns of the same synchronous recipient. In this situation, the smaller blastocysts were less viable than were the larger littermates. However, when transferred to less advanced recipients, the smaller blastocysts survived as well as the larger blastocysts. These results support the concept that most pig blastocysts are initially viable, but the less developed blastocysts are more susceptible to an advanced uterine environment than are the more advanced ones. Thus, uterine advancement on d 11 was embryocidal to less developed embryos (Morgan et al., 1987; Geisert et al., 1991). In addition, d 11 embryos from normally fed animals produced more estrogen than those from gilts fed a high-energy diet (Cassar et al., 1994), suggesting that high estrogen productivity may play a role in embryonic survival.

Investigations of Xie et al. (1990b) suggest that the more developed blastocysts within a litter are initially localized in a uterine microenvironment with more advanced secretions than in those areas of the uterus adjacent to less developed blastocysts. The 'advancing' uterine environment eventually extends to adjacent portions of the uterus and becomes too advanced, and thus too asynchronous, relative to lesser-developed blastocysts, and will ultimately cause the demise of the smaller blastocysts. Furthermore, the exogenous administration of estrogen on d 9 and 10 also results in the death of the blastocysts 4 to 6 days later (Morgan et al., 1987; Gries et al., 1989). The effects of

estrogen on embryonic survival on d 12 of pregnancy emphasize the importance of developmental uniformity in increasing embryonic survival rate and litter size, as reported to occur in more prolific Chinese Meishan pigs (Bazer et al., 1988). Meishan pigs have a slower rate of development from the 4-cell to morula stage, which may be advantageous to the embryos because it is thought that at this stage they are less sensitive to uterine changes (Youngs et al., 1993). From then on, embryo development is quicker and on d 12, embryos recovered from Meishan gilts contain more cells than those recovered from Large White × Landrace gilts (cited by Ashworth and Pickard, 1998). Furthermore, no differences in the within-litter variability in radiolabelled protein or estradiol-17beta secretion (Ashworth et al., 1997) per conceptus were found. However, it appears that the onset of conceptus secretion of estradiol-17beta occurs more synchronously in Meishan gilts (Pickard and Ashworth, 1995).

2.2.5. Within-litter diversity: follicular heterogeneity

A series of experiments have suggested that events during oogenesis may cause the variance in oocyte maturity before and in embryonic development after ovulation and subsequently influence survival of swine embryos (Pope et al., 1990, Pope, 1994; Van der Lende et al., 1994; Hunter and Picton, 1995). Components of this hypothesis include the following: (1) more-developed embryos within a litter have a better chance of survival than lesser-developed embryos (Pope et al., 1982b; Morgan et al., 1987); (2) more-developed embryos synthesize estradiol sooner than their contemporaries (Ford et al., 1982; Geisert et al., 1982a; Pope, 1988); (3) within a litter, the less-developed embryos are more sensitive to uterine advancement than the more-developed embryos (Wilde et al., 1988); (4) destruction of later-ovulating follicles eliminates the lesser-developed embryos normally present on d 11 (Pope et al., 1988); (5) diversity of embryo development is not related to the duration of ovulation (Soede et al., 1992); (6) increased dietary energy density after mating, which is detrimental to embryonic survival at d 28 of pregnancy (Jindal et al., 1997), is not found to influence the rate and uniformity of embryonic development, compared with normally fed animals (Cassar et al., 1994); and (7) induced ovulation by hCG injection was associated with reduced embryo survival on d 30 of pregnancy in Meishan gilts (Hunter and Picton, 1995).

There appears to be a considerable range in the morphological and biochemical development (hCG binding, follicular estradiol and testosterone concentrations) of preovulatory follicles in the pig. Foxcroft and Hunter (1985) were the first to discuss the physiological significance of follicular heterogeneity. In cyclic gilts, and in lactating and weaned sows, considerable variation in the development of preovulatory follicles has been reported, and at the assumed time of ovulation 36-40 h after the LH surge, all follicles will not be at an identical stage of maturity (Foxcroft et al., 1987; Grant et al., 1989; Hunter and Weisak, 1990; Zak et al., 1997b). In cyclic gilts, Pope et al. (1988) reported that approximately 70% of all ovulations occur within a 2 to 4 period and destruction of unovulated follicles after this time gave rise to a more uniform population of blastocysts at d 11 of gestation. A further comparison of oocyte maturational distribution before ovulation with zygotic maturation in gilts revealed that skewedness of oocyte development continued into the zygotic population (Xie et al., 1990a). In another experiment, Xie et al. (1990b) removed late-ovulating follicles by electrocautery and suggested on the basis of greater uniformity of the surviving embryos that late ovulating follicles gave rise to less well developed embryos in early gestation. Hence, the asynchronous development of the early embryo (Pope et al., 1986), and the subsequent occurrence of embryonic mortality, may be a consequence of the initial range of follicular development within the selected ovulatory population (Grant et al., 1989).

In the highly prolific Meishan pig breed, more oocytes collected after the endogenous LH surge had matured to metaphase II of meiosis than had comparable oocytes retrieved from Large White animals and this may contribute to the prolificacy of the breed (Faillace and Hunter, 1994). Again, an effect of feed restriction during lactation in primiparous sows on follicular development (Quesnel et al., 1998) and oocyte maturation (Zak et al., 1997b) has been reported with more oocytes recovered from sows fed to appetite maturing to metaphase II of meiosis *in vitro* than those from feed-restricted sows. It can be concluded that differences in the maturation of the follicle and oocyte in the period before ovulation might contribute to subsequent differences in embryonic survival. Therefore, any factors including feeding management that affect follicular development, may affect the maturation of oocytes and embryonic survival.

Given these associations, an understanding of follicular maturation in the pig is also essential for the development of experimental approaches for studying the mechanism mediating embryonic loss in swine.

2.3. Follicular development and maturation

The basic function of the ovulatory follicle is to produce a fertilizable oocyte, and to function as an endocrine gland during oocyte maturation and after ovulation through transformation into a functional corpus luteum. This processing is under the primary control of two pituitary hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

2.3.1. Gonadotropins sustain follicular growth and steroidogenesis

Follicular development includes two stages. The initial development of primordial follicles appears to be gonadotropin independent. Data obtained in other species using models in which gonadotropin concentrations are minimal such as hypophysectomy in sheep (Dufour et al., 1979), *in vitro* culture of bovine follicles in the absence of gonadotropins (Wandji et al., 1996), and knock-out of the FSH beta gene in mice (Kumar et al., 1997), suggest that local autocrine and paracrine regulation is of key importance at this stage. Using hypophysectomy and the gonadotropin releasing hormone (GnRH) antagonist Antarelix; which blocks pulsatile LH secretion but does not affect FSH, Driancourt et al. (1995) demonstrated that development of 0.19-1.1 mm size follicles in gilts was not affected by treatments, development of 1.1-2 mm follicles was affected by hypophysectomy but not by Antarelix treatment, and development of >2mm follicles was affected by both hypophysectomy and Antarelix, compared to intact gilts. They concluded that the growth of 0.19 to 1.1-mm size follicles is gonadotropin-independent, from 1.1 to 2 mm growth is FSH-dependent, and growth of follicles larger than 2 mm is LH-dependent.

From a biochemical perspective, as follicles develop 2 to 3 layers of granulosa cells, and the theca interna and externa cells begin to differentiate, FSH receptors appear on granulosa cells (Nakano et al., 1977), and follicular development enters the second stage and becomes gonadotropin-dependent. FSH is the critical stimulator of porcine follicular development (Ainsworth et al., 1990). As the follicles develop into medium and

large size follicles, functional FSH receptors (LaBarbera, 1994), receptor mRNA expression (Yuan et al., 1996; Liu et al., 1998) and FSH-responsive adenylic cyclase activity (LaBarbera, 1994) increase and then decrease. FSH itself causes down-regulation of FSH receptors (Sites et al., 1994). LH receptors are located in the theca cells of follicles greater than 100 μm in diameter (Channing and Kammerman, 1973; Meduri et al., 1992). As follicles develop into large antral, and especially into preovulatory follicles, LH receptor mRNA expression increases. By this stage, two distinct theca regions could be distinguished: the externa in which LH receptors are highly expressed and the interna, which is devoid of LH receptors but is still steroidogenic (Meduri et al., 1996). Granulosa cells possess LH receptors only at the antral stage of follicle development. Both mRNA expression for the LH receptor, and the number of functional receptors are maximal on d 20 of the estrous cycle (Hsueh et al., 1984; Yuan et al., 1996; Liu et al., 1998). Ovulation does not change the distribution of LH receptors (Meduri et al., 1996), but the LH receptor number and affinity decline to their lowest level after ovulation, compared with granulosa cells before ovulation and luteal cells at the mid-luteal phase of the cycle (Meduri et al., 1996; Gebarowska et al., 1997; Liu et al., 1998).

The pattern of steroidogenesis in pig follicles is consistent with the two-cell-two-gonadotropin theory of steroidogenesis, in that androgen produced by the theca in response to LH is aromatized to estrogen by the granulosa cells in response to FSH (Foxcroft and Hunter, 1985; Gore-Langton and Armstrong, 1988; Hillier et al., 1994; Schoham and Schachter, 1996). Both FSH and LH promote mRNA synthesis for Cholesterol Side-Chain Cleavage Cytochrome (P450scc), aromatase (P450arom), and 3beta-Hydroxysteroid-Dehydrogenase (3beta-HSD), increase steroidogenesis, and stimulate follicular growth and thus the number of steroidogenic cells (Mulheron et al., 1990). 17alpha-Hydroxylase-c17-20 lyase (P450c17) activity is expressed exclusively in the theca cells and regulated by LH and local factors (Hillier et al., 1994). Thus androgen is synthesized only by theca cells. Estrogen synthesis is dependent on the availability of androgen (Gore-Langton and Armstrong, 1988). Evans et al. (1981) were the first to show that porcine theca cells have aromatase activity and can synthesize estradiol. Using immuno-histochemical methods, both Conley et al. (1994) and Meduri et al. (1996) confirmed that theca cells express P450c17, P450arom, P450scc, and 3beta-HSD,

whereas granulosa cells express only P450arom and a low level of P450scc. Thus, in the pig, the theca interna has the capacity to produce estradiol. The expression of theca P450c17, theca P450arom and granulosa P450arom is closely correlated with follicular fluid levels of estradiol, androstenedione and testosterone during preovulatory follicular development (Conley et al., 1994). In other species, granulosa cells are still the main source of estradiol, for the aromatase activity of the granulosa cell layer is at least 700-fold greater than that of the theca layer (Hillier et al., 1981).

2.3.2. Follicle recruitment

Recruitment is the time at which a pool of growing follicles begin to grow rapidly and if "selected' will progress to ovulate (Armstrong and Webb, 1997). The length of estrous cycle in pigs is 21 days on average (see Figure 2.1). It has been established that final follicle recruitment into the preovulatory pool occurs between d 14 and 16 of the cycle in pigs at a size of about 3-5 mm in diameter (Foxcroft and Hunter, 1985). Recruitment is not a random or isolated phenomenon; on the contrary, follicles seem to be recruited as groups or cohorts, suggesting that they have received a signal that allows them to continue growth and development rather than regress. It is likely that both FSH and LH are necessary for recruitment (Foxcroft et al., 1994). Increased follicular development within the proliferating pool can be achieved in the sow during lactation through prolonged exposure of the ovaries to raised levels of FSH (Britt et al., 1985). In the cyclic pig, at the time of recruitment around d 14 and 16, the rate of LH episodic secretion increases as progesterone concentrations decline, while FSH concentrations drop (Hasegawa et al., 1988), coincident with luteolysis of the corpora lutea. In weaned sows, in which there is active LH secretion, many of the larger follicles in the hierarchy can be estrogenic within 48 h after weaning (Britt et al., 1985; Foxcroft et al., 1987). All these studies suggest that the robust increase in LH pulsatile secretion is a probable signal for initiation of follicle recruitment and maturation, with or without a parallel increase in plasma FSH concentrations.

2.3.3. Follicle selection

The selection procedure, which determines the final population of preovulatory follicles, occurs between d 16 to 20 of the cycle. This process involves both atresia and a

concomitant block to the recruitment of new follicles into this pool by the suppression of FSH secretion via the feedback of estradiol and inhibin (Guthrie et al., 1995). One of the most important events during selection is the appearance of aromatase activity in granulosa cells (Gougeon, 1996). Only at d 20 of the cycle is a final subset of about 15 preovulatory follicles apparent in the gilt (Hunter et al., 1989). It has been demonstrated that in terms of stimulating granulosa cell aromatase activity, preovulatory follicles are at least 10 times more sensitive to FSH than small follicles (Harlow et al., 1986). FSH is important in follicle growth in the pig, but as suggested by Foxcroft (1992) and Foxcroft et al. (1995), the pattern of LH secretion may play an active role in preovulatory follicle selection by altering the responsiveness of the growing follicle to FSH. As discussed earlier, GnRH antagonist treatment in gilts further confirmed the requirement for LH in final follicular maturation (Driancourt et al., 1995). Data from other species suggest that in the later stage of selection, increased numbers of gonadotropin receptors, and increased production of intraovarian peptides (such as Inhibin) and growth factors (such as IGF-I, TGF-β), contribute to enhanced follicular responsiveness to gonadotropins (Gougeon, 1996; Armstrong and Webb, 1997). The next sections review similar evidence from studies in swine.

2.3.4. Local regulation of folliculogenesis

2.3.4.1 Inhibin-related peptides

The family of inhibin-related proteins includes inhibin, activin and follistatin. By the beginning of 90's, it had already been established that inhibin, activin and follistatin, synthesized and secreted by follicular granulosa cells, exert local autocrine/paracrine actions to modulate follicular maturation and steroidogenesis (Knight, 1996). Receptors for activin (reviewed by Mathews, 1994) and its receptor mRNA (Cameron et al., 1994) have been identified on granulosa cells, theca cells and oocytes, consistent with local actions on these cell types.

Inhibin is a dimeric protein composed of an alpha (18kDa) and a beta (14kDa) subunit. Two distinct beta subunits are recognized and characterize two different forms of inhibin (A and B). Both forms of inhibin have an inhibitory effect on pituitary FSH release (see Knight, 1996, for review). Activin opposes the action of inhibin to stimulate

pituitary FSH secretion and FSH beta mRNA accumulation (Vale et al., 1986). Three forms of activin were isolated from pig follicular fluid, referred to as activin A, AB and B (Nakamura et al., 1992). Inhibin and activin are synthesized predominantly by granulosa cells of developing follicles (Woodruff and Mather, 1995), but expression of inhibin and activin alpha and beta subunit mRNAs and protein has also been detected in rat ovarian theca cells (Jin et al., 1993). Studies in rat showed that the expression of the mRNAs that encode inhibin is modulated by a wide variety of growth factors. Insulin-like growth factor -I (IGF-I) (Carson et al., 1989), transforming growth factor-beta (TGFbeta) (LaPolt et al., 1989) and activin (Xiao et al., 1992) all increase inhibin production, while epidermal growth factor (EGF) and TGFalpha (Zhiwen et al., 1987) decrease inhibin levels. There have been very few reports on the effects of locally produced factors on granulosa cell expression of activin. The third member of the family, follistatin, characterized as a 35-kilodalton monomeric, cysteine-rich, polypeptide and structurally unrelated to inhibin, suppresses pituitary FSH release in a similar manner to inhibin. Six molecular forms of follistatin exist in porcine ovaries (Sugino et al., 1993). Follistatin can neutralize activin bioactivity in rats (Xiao et al., 1992; Li et al., 1995) by inhibiting the binding of activin to all known activin type II receptors (De Winter et al., 1996). Follistatin mRNA and protein are predominantly expressed in rat granulosa cells of follicle, with the level of expression increasing as follicular maturation progresses (Nakatani et al., 1991), and their production is under the control of FSH (Michel et al., 1992). EGF stimulates the expression of follistatin mRNA in pigs and may involve a protein kinase C pathway (Lindsell et al., 1993).

Inhibin is the most clearly identified ovarian factor controlling FSH release from the pituitary in pigs and plasma inhibin and FSH are normally inversely related (Hasegawa et al., 1988; Taya et al., 1991). Active immunization of gilts (Brown et al., 1990; King et al., 1995), or ewes (Dietrich et al., 1995) against inhibin increased ovulation rate and was often associated with increased FSH concentrations. However, activin can promote FSH release, acting as a functional antagonist to inhibin. Treatment of granulosa cells from diethylstilboestrol-treated rats (FSH-suppressed) with activin alone can up-regulate FSH receptor expression, enhance responsiveness to FSH and LH (Xiao et al., 1992), and increase expression of inhibin alpha subunit (LaPolt et al., 1989)

and follistatin mRNA (Michel et al., 1992). In the presence of FSH, activin can enhance aromatase activity, estrogen production and progesterone secretion (Miro et al., 1991), and increase expression of FSH receptor (Xiao and Findlay, 1991), FSH-induced granulosa cell proliferation (Miro and Hillier, 1996) and follistatin (Michel et al., 1992). In the presence of FSH, activin also causes primary follicles to develop into preovulatory follicles *in vitro* (Li et al., 1995). However, in contrast to these findings, Chang et al. (1996) and Ford and Howard (1997) reported that activin-A inhibits FSH-induced and basal steroidogenesis and aromatase activity in porcine granulosa cells. In addition, activin is able to inhibit both a spontaneous and LH/hCG-induced increase in progesterone output by more fully differentiated bovine granulosa cells (Shukovski et al., 1991). This action is reversed by follistatin. Based on these data, it can be concluded that biologically active activin would serve as a negative modulator of steroidogenesis in porcine antral follicles and could play a role in follicular atresia (Chang et al., 1996; Ford and Howard, 1997). Its differential role in the follicle may depend on cell type, presence of other growth factors, and specific intra- and extra-cellular environments (Chang et al., 1996).

The ability of activin to promote FSH receptor expression in undifferentiated rodent granulosa cells may be of particular significance, since this could explain how a small preantral follicle progresses from a gonadotropin-independent to a gonadotropin-dependent stage of development (see Knight, 1996 for review). Having acquired FSH receptors, further growth and differentiation of granulosa cells through to the preovulatory stage would be predominantly driven by FSH acting in synergy with activin, but also modulated by follistatin and other local factors (i.e. steroids, IGFs). Once follicles are selected, activin production declines, as inhibin levels increase (Hillier, 1991). Inhibin then may promote further development of the follicle to the preovulatory stage in view of its ability to enhance LH-induced theca androgen production, thereby increasing the substrate for granulosa cell estrogen production (Hsueh et al., 1987).

2.3.4.2. Insulin-like growth factor-I system

The ovarian IGF-I system, comprised of IGF-I peptide, IGF-I receptor, IGF binding protein (IGFBP) and protease degrading the binding protein, plays an important role in the process of follicular development and steroidogenesis (Hammond et al., 1993;

Yoshimura, 1998). The presence of IGF-1 and its receptors in the pig (Veldhuis and Furlanetto, 1985), as well as IGF-1 mRNA and IGF-1 receptor mRNA (Samaras et al., 1993; Zhou et al., 1996) has identified granulosa cells as a major site of IGF-I synthesis and action. The pattern of IGF-1 receptor gene expression is heavily and selectively concentrated in granulosa cells. IGF-1 receptor mRNA levels are highest in healthy growing and dominant porcine follicle granulosa cells and reduced in atretic follicles regardless of follicles size (Zhou et al., 1996), suggesting a loss of IGF-1 effect due to receptor down-regulation may be involved in follicular atresia in the pig.

Insulin-like growth factor I promotes both the replication and differentiation of granulosa cells and affects virtually all aspects of granulosa cell function, including enhancement of gonadotropin-stimulated production of progesterone, estrogen and proteoglycans, and LH receptor induction (reviewed by Tonetta and diZerega, 1990). IGF-I actions appear to occur almost exclusively in synergy with gonadotropins. IGF-I amplification of FSH action at the level of the granulosa cell includes stimulation of aromatase activity and mRNA expression (Mason et al., 1993), an increase in 3beta-HSD mRNA (Erickson et al., 1991) and cytochrome P450 side chain cleavage enzyme expression (Urban et al., 1990, 1994). Therefore, IGF-I enhances FSH-induced progesterone (Erickson et al., 1991; Urban et al., 1990, 1994) and estradiol production (Xu et al., 1997). This amplifying property of IGF-I is attributable to multiple actions, including enhanced generation of cAMP (Adashi et al., 1986), enhanced uptake of lipoproteins (Veldhuis et al., 1987), induction of LH receptors (Adashi et al., 1985), and stimulation of steroidogenic enzyme activity and mRNA expression (Urban et al., 1994; deMoura et al., 1997). *In vitro* studies also show that IGF-1 suppresses porcine granulosa cell apoptosis (Guthrie et al., 1998) and follicular atresia (Chun et al., 1996) as effectively as gonadotropins.

IGF-I acts in a synergistic manner with LH in theca-interstitial cells to increase androgen biosynthesis (Magoffin et al., 1990; Spicer et al., 1997). This effect may be caused by the ability of IGF-I to increase cholesterol-side-chain cleavage and 17alpha-hydroxylase mRNA and protein content in theca-interstitial cells above those stimulated by LH alone (Spicer et al., 1997), thereby enhancing androgen precursor in the theca as the substrate for IGF-I-stimulated granulosa aromatase in the production of estradiol.

IGF-I also increased the hCG-binding capacity of the theca-interstitial cells (Cara et al., 1990), suggesting that the synergistic interaction of IGF-I with LH produces multiple effects. It is this amplifying property of IGF-I, coupled with its selective expression in healthy (but not atretic) follicles (Oliver et al., 1989), that may underlie its purported ability to initiate and maintain the process of follicular development (Adashi, 1994).

IGF-1 follicular fluid concentration increases during spontaneous and gonadotropin- and GH-induced development during the follicular phase (Hammond et al., 1993; Samaras et al., 1994). A significant correlation of follicular fluid IGF-1 concentration with follicular size and estradiol concentrations in the follicular fluid has also been reported (Sarmaras et al., 1993). Similar results have been reported during follicular development in the weaned sow (Howard and Ford, 1992).

IGFBPs play a significant role in regulating the IGF enhancement of gonadotropin actions on granulosa cells. IGFBPs have been shown to neutralize IGF-I action in the ovary (Bicsak et al., 1990; Chun et al., 1996). IGFBPs could inhibit IGF-I-stimulated estradiol and progesterone production by human granulosa cells (Mason et al., 1992). The effects of IGFBPs therefore considered to be antigonadotropic, achieved by their sequestering granulosa cell-derived IGF-I, thereby limiting the availability of IGF-I to synergize with gonadotropins acting on the ovary (Shimasaki et al., 1990; Yoshimura et al., 1996). The pig ovary expresses IGFBP-2, -3, -4, and -5, with granulosa cell IGFBP-2 located by *in situ* hybridization to small follicles and IGFBP-4 to large follicles (Guthrie et al., 1995; Zhou et al., 1996). IGFBP-2 mRNA and protein decreased with porcine follicle development, while IGF-I increased (Guthrie et al., 1995). Cultured porcine granulosa cells elaborate all the IGFBPs, with production of IGFBP-3 and -5 stimulated by IGF-I and IGFBP-4 by forskolin (Leighton et al., 1994), while FSH inhibits IGFBP-2, -3 and -5 mRNA and protein production (Grimes and Hammond, 1992; Grimes et al., 1992). In porcine follicular fluid, follicular growth is accompanied by a slight increase in IGFBP-3 and a decrease in IGFBP-2 and -4. At the preovulatory stage, IGFBP-4 and -5 are undetectable in follicular fluid (Mondschein et al., 1991; Howard and Ford, 1992; Grimes et al., 1994). Atresia is associated with a marked increase in intrafollicular levels of IGFBP-2 and IGFBP-4 (Grimes et al., 1994; Howard and Ford, 1992; Guthrie et al., 1995).

Proteolysis of IGFBPs by a variety of proteases appears to be a fundamental mechanism in regulating the bioavailability of IGF-1 (Besnard et al., 1997). Grimes and Hammond (1994) demonstrated that these proteases are derived from granulosa cells. In the rat, granulosa cell-derived IGFBP-4 and -5 protease activity was induced by FSH (Fielder et al., 1993) and in pigs, follicular growth was characterized by a dramatic increase in proteolytic activity (Besnard et al., 1997). Interestingly, in the more prolific Chinese Meishan breed, protease activity (metalloproteinases, MMP-2 and MMP-9) was higher in the luteinizing follicles, but the tissue inhibitor of metalloproteinase activity was lower, compared to Large White pigs (Driancourt et al., 1998).

2.3.4.3. Insulin

Insulin also affects follicular development. It could support follicular development by reducing follicular atresia (Meurer et al., 1991a,b; Matamoros et al., 1990, 1991) and by suppressing follicular apoptosis (Purvis et al., 1997). *In vivo* studies demonstrated that exogenous insulin treatment for 4 days in gonadotropin-treated prepubertal gilts increased the number of follicles ≤3 mm (Matamoros et al., 1991), and maintained the 4-6 mm size follicle population in cyclic gilts when insulin treatment began on d 15 of the cycle (Matamoros et al., 1990). In primiparous sows, insulin treatment for 3 days after weaning increased the number of large follicles (Whitley et al., 1998a,b). Conversely, withdrawal of insulin in Streptozotocin-induced-diabetic pigs reduced follicle diameter and estradiol production, and increased atresia (Cox et al., 1994; Edwards et al., 1996). Insulin treatment in feed-restricted gilts during the late luteal phase of the estrous cycle increased peak estradiol concentrations in the follicular phase and the magnitude of the preovulatory LH surge (Almeida et al., 2000b), suggesting that insulin most likely enhanced follicular development in these animals. *In vitro* studies have shown that insulin could inhibit IGFBP production (Poretsky et al., 1996), act synergistically with FSH to increase 3 beta-HSD activity and progesterone production in human granulosa cells (McGee et al., 1995; Willis et al., 1998) and stimulate DNA synthesis of theca-interstitial cells (Duleba et al., 1997, 1998).

At the cellular level, porcine granulosa cells possess insulin receptors (Rein and Schomberg, 1982). Insulin can both interact with its own receptor and cross-react with

IGF-I receptors to regulate folliculogenesis (McArdle et al., 1991). However, several lines of evidence suggest that insulin receptors mediate insulin's actions. *In vitro* studies in human granulosa cells showed that insulin-stimulated steroid production could be inhibited by anti-insulin receptor antibodies, but not by antibodies against the IGF-I receptors (Willis and Frank., 1995). Nestler et al. (1998) demonstrated that insulin stimulation of testosterone production could not be inhibited by an antibody against IGF-I receptor in cultured human theca cells, suggesting that this effect of insulin was also mediated by the insulin receptor.

2.3.5. Follicle development during lactation and after weaning

Ovarian function throughout lactation has been examined by several workers (Palmer et al., 1965; Sesti and Britt, 1994) and folliculogenesis is considered to be relatively quiescent. Sesti and Britt (1994) examined follicular development in the early stage of lactation and found that all size classes (small: <3mm diameter; medium: 4-6mm; large: >6mm) of follicles exist during the first 24 h of lactation, but at d 7, only small follicles are found in the ovaries. Observation made by Edwards (1982) also showed that following parturition and the initiation of lactation the ovaries remain dormant for about 10 days. As lactation progresses, suckling intensity decreases (Varley and Foxcroft, 1990), the size of the readily-releasable pool of GnRH (Sesti and Britt, 1993b), and LH and FSH (Sesti and Britt, 1993a) increase, small follicles may begin to develop, and the population of medium and large follicles increases from mid (d 14)- to late (d 28)-lactation (Sesti and Britt, 1993b; Figure 2.2), although none attains the size of >6mm diameter (Britt et al., 1985).

Weaning results in a rapid increase in the number of medium to large sized follicles (Lauderdale et al., 1965; Foxcroft et al., 1987; Quesnel et al., 1998). If the average weaning-to-estrus interval is 5 days, the time of weaning would be equivalent to d 16 in a cyclic animal, i.e. the time when follicles are being actively recruited into the preovulatory pool. In the pubertal gilt, Morbeck et al. (1992) estimated that a follicle took 14 days to grow from antrum formation to 3 mm in diameter and a further 5 days to become preovulatory in size. Thus follicles undergoing antrum formation during lactation constitute the preovulatory pool from which ovulatory follicles are selected after weaning. Nutritional status of the animal during lactation may therefore have a profound

effect on follicular development and potentially on oocyte maturation (Foxcroft et al., 1995). Support for this hypothesis came from the studies of Zak et al. (1997b) and Yang (1998). The effects of feed restriction during 28-d lactation on follicular development at weaning and 48 h after weaning in primiparous sows were also studied by Quesnel et al. (1998). Compared to full-fed sows, 50% of ad libitum feed intake affected several characteristics of follicular development, including size of the 10 largest follicles, follicular fluid volume, number of large follicles (> 4mm in diameter), and had a small effect on follicular fluid IGF-I concentrations. Thus, even during lactation, nutritional status retards the development of follicles within the proliferating pool.

2.4. Luteal function and its regulation

2.4.1. Luteinization and associated changes

The formation of the corpus luteum (CL) represents a continuation of follicular maturation and the CL forms after ovulation from the theca, granulosa and associated blood cells (see Niswender et al., 1994 for review; Meduri et al., 1996). Substantial biochemical changes are associated with the luteinization of theca interna and granulosa cells. After the LH surge, but prior to ovulation, there is a temporary decrease in mRNA for P450scc and 3beta-HSD (Conley et al., 1995). This is followed by increases in mRNA and enzyme activity for P450scc and 3beta-HSD after ovulation and luteal formation (Conley et al., 1995; Meduri et al., 1996). There is decrease in mRNA expression and secreted protein for 17alpha-hydroxylase cytochrome P450 in rats, which catalyzes conversion of pregnolone or progesterone to androgen, and whilst abundant in preovulatory follicles is low in the CL (Hedin et al., 1987). Aromatase cytochrome P450 enzyme mRNA expression and protein also decrease rapidly after the LH surge (rat: Hickey et al., 1988; pig: Meduri et a., 1996). Therefore, luteinization results in decreases in androgen and estrogen production and an increase in progesterone production. However, mechanisms associated with lutenization are not dependent on follicular rupture and ovulation does not guarantee normal luteal development and function (reviewed by Smith et al., 1994b).

In the immediate postovulatory period, the distribution of LH receptors and steroidogenic enzymes does not change (Muderi et al., 1996). However, LH receptors are

down-regulated due to internalization of occupied receptors and reduced expression of genes encoding the receptor (Meduri et al., 1996; Gebarowska et al., 1997). Data of Meduri et al. (1996) suggest that in the theca external cells, the LH receptors undergo down-regulation after ovulation, then recover and persist during the further development of the corpus luteum. The internal theca cells, which are LH receptor negative in preovulatory follicles, remain so during luteal development. The LH receptors on the granulosa-derived cells are also down-regulated, but do not recover at further stages of corpus luteum development. In addition, the binding affinity of LH receptors on luteal cells immediately post-ovulation is lower than that on granulosa cells (Gebarowska et al., 1997).

2.4.2. Luteal cell types

The corpus luteum consists of a heterogeneous population of cells that have distinct morphological, endocrine, and biochemical properties. Cell types that have been identified in the corpora lutea include steroidogenic (including small and large luteal cells) (Lemon and Loir, 1977) and non-steroidogenic cells (such as fibroblasts and endothelial cells).

The two steroid-producing cell subpopulations, of which small and large luteal cells are derived from theca and granulosa cells, respectively (Meduri et al., 1996), differ in a number of characteristics, with cell size being the most obvious (Pate, 1996). Morphometric studies revealed that small luteal cells are the most abundant cell type in the corpus luteum of bovine and human (Lei et al., 1991). The ultrastructure of these two cell types is compatible with that of cells with active synthesis of steroids (small and large cells) and proteins (large cells). Thus, large cells present numerous mitochondria, a well developed smooth and rough endoplasmic reticulum, and secretory granules bound to the endoplasmic membrane, whose content is exocytosed in relation to the rate of *in vivo* and *in vitro* progesterone production (Smith, 1986; Yuan et al., 1993). In the small cells, there are numerous lipid droplets and abundant smooth endoplasmic reticulum (Vega and Devoto, 1997).

Among other cells that are not predominantly steroidogenic, macrophages are particularly important because of their capacity to secrete growth factors and cytokines (see Pate, 1995 for review). Macrophages have been implicated in many functions of

corpora lutea, such as angiogenesis, luteolysis, and progesterone biosynthesis (Behrman and Preston 1989; Vega et al., 1994). Endothelial cells also play a very important role in the formation of corpus luteum (Pate, 1996).

Therefore, interactions between steroidogenic and non-steroidogenic cells, and between large and small luteal cells, exist within corpus luteum and are important for growth, differentiation and function of the luteal tissue.

2.4.3. Interactions among luteal cells

2.4.3.1. Large- and small- luteal cell interactions

There are distinct differences in the ability of small and large cells to secrete progesterone in the presence or absence of LH. Basal progesterone production *in vitro* was lower in small compared to large cells, which may be associated with the difference in ultrastructure (Hunter, 1981; Yuan et al., 1993; Richards et al., 1994). LH receptors are found on small luteal cells only (Meduri et al., 1992, 1996), whereas PGE receptors are present primarily on large luteal cells (Feng and Almond, 1996). Consequently, LH stimulates progesterone secretion in small luteal cells only (Richards et al., 1994; Feng and Almond, 1998). Conversely, PGE2 only stimulates progesterone secretion by large cells (Richards et al., 1994).

Given that the two luteal cell types are functionally distinct, it seems reasonable that interactions between these cells might occur during LH stimulation of progesterone production. Lemon and Mauleon (1982) first demonstrated a synergistic effect when small and large luteal cells were incubated together, and further showed that the effect was due to small cell stimulation of large cell steroidogenesis. Luteal cells secrete a wide variety of compounds that may act in a paracrine manner, including prostaglandins, peptides, growth factors and steroids (Pate, 1996).

Prostaglandins: Porcine corpora lutea produce prostaglandin E2 and F2alpha (Watson and Patek, 1979) and possesses prostaglandin receptors (Feng and Almond, 1996, 1999). PGE2 and PGF2alpha generally exert opposite effects on corpus luteum function *in vivo*. Exogenous PGF2alpha consistently induces a precipitous premature luteal regression (Gadsby et al., 1991), whereas PGE2 administration tends to prolong the luteal phase (Akinlosotu et al., 1988). *In vitro*, PGE alone stimulates progesterone

production in a dose dependent manner (Wiesak et al., 1992; Gregoraszczuk and Michas, 1999). However, PGE + LH caused inhibition of progesterone production (Wiesak et al., 1992), which suggests PGE is involved in the autocrine control of luteal function.

IGF-I system: A complete intraovarian IGF system, including IGF-I, IGF receptors and IGFBP2-5, is present in pig corpora lutea. But variable expression between large and small luteal cell exists, with IGF-I and IGFBP-2 and -4 being expressed in both cell types, while mRNAs for IGFBP-3 and -5 are predominantly expressed in small cells (Gadsby et al., 1996). IGF-I receptor binding activity (Parmer et al., 1991; Sauerwein et al., 1992) and mRNA (Parmer et al., 1991) have been demonstrated in the rat and bovine corpus luteum. In rat, it has been found that large, but not small, luteal cells expressed the IGF receptor, IGF-stimulated steroidogenesis (Parmer et al., 1991), and IGF-I-enhanced lipoprotein utilization for steroidogenesis (Pate, 1996). The study by Gadsby et al. (1996) also showed that progesterone secretion by large, but not small, porcine luteal cells was increased in a dose-dependent manner in response to exogenous IGF-I, suggesting that, as in the rat, large luteal cells are probably the cellular sites of the IGF-I receptor and IGF-I action. *In vitro* studies showed that IGF-I stimulates luteal progesterone (Urban et al., 1990; Constantino et al., 1991; Parmer et al., 1991; Huang et al., 1992; Yuan and Lucy, 1996; Balasubramanian et al., 1997), and relaxin (Ohleth and Bagnell, 1999) secretion, and increased both steroidogenic acute regulatory (StAR) protein and P450scc mRNA expression (Pescador et al., 1999).

Progesterone: Progesterone is believed to be a luteotropic factor. Using immuno-cytochemical techniques, it was demonstrated that corpus luteum expresses specific progesterone receptors in the monkey (Stouffer et al., 1993). Further data from Stoufer et al. (1993) showed that progesterone receptors are similar to those in other tissues of the reproductive tract and are functional in terms of the ability to bind progesterone. Progesterone stimulates the activity of 3beta-HSD and cholesterol esterase in ovarian cells (rat: Ruiz et al., 1985; pig: Tonetta, 1987). In cultured bovine luteal cells, progesterone decreased basal production of PGF2alpha and PGI2, inhibited cytokine-stimulated prostaglandin synthesis, and increased LH receptors (Pate, 1988, and 1995).

Estradiol: The reverse transcription-polymerase chain reaction (RT-PCR) technique has demonstrated the high expression of estradiol receptor gene in the pig

(Wuttke et al., 1997), which suggests an important regulating mechanism by estradiol. The developing corpus luteum has high aromatase activity (Meduri et al., 1996) and produces estradiol. *In vitro*, estradiol stimulates progesterone release from pig luteal cells (Pitzel et a., 1993a,b). *In vivo*, estradiol increases luteal progesterone production and lengthens the luteal phase of gilts (Conley and Ford, 1989; Pusateri et al., 1996) and the intraluteal application of estradiol stimulates progesterone release in sows (Jarry et al., 1990).

2.4.3.2. Interactions between luteal cells and other cells

Immune cell and luteal cell interactions: Immune cells and luteal cells likely communicate via secreted products, which includes cytokines, prostaglandins and growth factors. Immune cells produce several cytokines, including interleukin 1beta (IL-1beta), TNF-alpha and interferon-γ (IFN-γ), which have been shown to affect progesterone and prostaglandin production (Brannstrom and Norman, 1993; Pate 1996), and may be pivotal in tissue remodeling through the secretion of proteases during luteinization (Smith et al., 1994a).

Endothelial cell and luteal cell interactions: Vascularization is essential for corpus luteum formation and is regulated by vascular endothelial growth factors (VEGF). VEGF induces endothelial cell proliferation as well as angiogenesis, and increases capillary permeability. VEGF, and its mRNA are expressed in corpora lutea (Yan et al., 1993; Yamamoto et al., 1997) and three receptors for VEGF have been identified (KDR, flt-1, flt-4) (Yan et al., 1998). Further study showed that VEGF-receptor expression was restricted to endothelial cells. Therefore, VEGF is a paracrine growth factor from luteal cells directed to endothelial cells. VEGF expression reflects luteal function and a positive linear correlation between VEGF mRNA with plasma progesterone levels was found (Doldi et al., 1997). The study by Ferrara et al. (1998) showed that treatment with truncated soluble Flt-1 receptors, which inhibit VEGF bioactivity, resulted in virtually complete suppression of corpus luteum angiogenesis in a rat model of hormonally-induced ovulation. This effect was associated with inhibition of corpus luteum development and progesterone release. Both gonadotropin and IGF-I enhance VEGF production by luteinized granulosa cells (Lee et al., 1997; Schams et al., 1999). On the

other hand, luteal cells produce tissue inhibitors of metalloproteinases (Smith et al., 1994b), which may regulate endothelial cell migration during the angiogenic process (Smith et al., 1994a).

2.4.4. Progesterone synthesis

Biochemically, steroid hormone biosynthesis is modulated by cholesterol availability and expression of specific steroidogenic enzymes (Carr, 1998). The preovulatory LH surge initiates distinct changes in both expression and regulation of steroidogenic proteins/enzymes and is a key event in the luteinization process. StAR protein, P450scc, and 3beta-HSD are the three key protein/enzymes that are essential for normal luteal function (Carr, 1998).

StAR protein: The formation of pregnenolone from cholesterol, catalyzed by P450scc enzyme, is generally referred to as the rate-limiting step in the biosynthesis of progesterone (Miller, 1988). This step in the biosynthesis of steroid hormones is stimulated by tropic hormones but is ineffectual in augmenting steroidogenesis unless there is a concurrent increase in the translocation of cholesterol from the outer to the inner mitochondrial membranes (Simpson et al., 1978; Toaff et al., 1979). Consequently, effective cholesterol transport to the mitochondrial P450scc is believed to be a pivotal locus of steroidogenic regulation (Stocco, 1997, 1998). This process is mediated by a short-lived cycloheximide-sensitive protein (Simpson et al., 1978; Toaff et al., 1979; Miller, 1988), which was recently purified from mouse MA-10 Leydig tumor cell (Clark et al., 1994). It has been designated the steroidogenic acute regulatory protein (StAR). Considerable evidence suggests that the StAR protein mediates luteal function and is the rate-limiting step in progesterone biosynthesis (Stocco, 1997, 1998). StAR protein facilitates the transfer of cholesterol from the outer mitichondrial membrane to the inner mitochondrial membrane and the P450scc. It is believed that once converted to prognenolone, this more hydrophilic steroid is free to diffuse out of the mitochondria into the microsomes for further conversion to progesterone (see Figure 2.3).

P450scc: Conversion of cholesterol to pregnenolone is carried out in mitochondria by P450scc, part of a three-protein complex known as the cholesterol side-chain cleavage complex that includes adrenodoxin and adrenodoxin reductase, in addition

to the P450scc (Tuls et al., 1987). P450scc was expressed by all luteal cells and its concentrations increased rapidly after ovulation (Meduri et al., 1996).

3beta-HSD: 3beta-HSD catalyzes the conversion of prognenolone to progesterone. Similar to P450scc, 3beta-HSD was expressed by all luteal cells, with enhanced expression after ovulation (Meduri et al., 1996). Within bovine corpora lutea, 3beta-HSD mRNA and enzyme activity were increased throughout most of the estrous cycle, and then decreased during luteolysis (Couet et al., 1990), with 3beta-HSD enzyme activity four times greater in corpora lutea than in preovulatory follicles. Thus, the preovulatory gonadotropin surge results in acquisition of the 3beta-HSD activity within corpora lutea, which facilitates high rates of progesterone biosynthesis.

The expression and activity of these proteins/enzymes are regulated by gonadotropins and growth factors. It has been demonstrated that FSH and IGF-I alone (Balasubramanian et al., 1997; Pescador et al., 1997), or FSH with IGF-1 (Balasubramanian et al., 1997; Winters et al., 1998), FSH with LH (Chedrese et al., 1990) acting synergistically, induce amplification of StAR protein and P450scc mRNA and protein expression in pig granulosa cells. The synergistic regulation by LH, FSH and IGF-I of StAR mRNA and protein expression may prepare granulosa cells for the increased steroidogenic demand imposed during luteinization after the LH surge.

2.4.5. Progesterone as a mediator of embryonic survival

Although the mechanisms underlining nutritional effects on embryonic survival have not been fully elucidated, recent studies emphasize that progesterone production in early pregnancy may be a mediator of nutritional effects on embryonic survival (Parr et al., 1993; Ashworth et al., 1995; Jindal et al., 1996; Foxcroft, 1997; van den Brand et al., 2000b). Progesterone concentrations during early pregnancy can modify oviductal (Murray et al., 1995) and endometrial (Roberts et al., 1993) development and secretory activity, and be an important factor in determining the likelihood of an embryo remaining viable. Progesterone concentrations during early pregnancy in gilts were positively associated with embryonic survival (Pharazyn et al. 1991; Ashworth et al., 1995; Jindal et al., 1996) and a similar association was also established in the primiparous weaned sow (Clowes, 1994; Jindal, 1996; Zak et al., 1998; van den Brand et al., 2000b). Direct evidence to support the hypothesis that progesterone is a key regulator of embryonic

survival came from experiments in which exogenous progesterone treatment reversed detrimental effects of high plane feeding on embryo survival in sheep (Parr et al., 1987; Ashworth et al., 1989), since high feeding level after mating may increase the progesterone clearance rate (Parr et a., 1987). Likewise in gilts, with high ovulation rates but low embryonic survival as a consequence of *ad libitum* feeding before and after mating, supplementary progesterone after mating increased embryonic survival (Ashworth, 1991a; Jindal et al., 1997).

The time of rise in plasma progesterone concentrations after mating might be critical in influencing embryonic survival. In the study by Jindal et al. (1997), plasma progesterone concentration rose earlier after onset of estrus in gilts on a normal feed intake than the gilts on a high feed intake. An earlier increase in plasma progesterone concentrations in more prolific Chinese Meishan pigs than in Large White gilts has also been observed (Hunter et al., 1996) and may play a key role in the superior embryo survival of Meishan pigs. Similarly, a faster increase in plasma progesterone concentrations was observed in primiparous sows mated at the second estrus compared with the first estrus after weaning, and was associated with a significant increase in litter size born (Clowes et al., 1994). Recently, our laboratory demonstrated that feed restriction during the second week of the estrous cycle in gilts depressed plasma progesterone concentrations immediately after ovulation (Almeida et al., 2000a), resulted in a slower rise in progesterone in early pregnancy (Almeida et al., 2000b), and decreased embryonic survival rate at d 28 of pregnancy, compared with non-restricted gilts or gilts feed-restricted during the first week of the cycle. Therefore, one of the objectives of the research presented in this thesis was to investigate the progesterone-mediated mechanism for nutritional effects on embryonic survival rate by giving exogenous progesterone treatment in weaned sows, which is reported in Chapter 3, and to investigate luteal function in the feed-restricted gilt model, which was reported in Chapter 5.

2.4.6. Causes of inadequate luteal function

Due to their derivation from the preovulatory follicles, it is hardly surprising that factors influencing folliculogenesis may subsequently alter luteal function. Hunter and Wiesak (1990) reviewed evidence for heterogeneity in porcine CLs and suggested that it is likely a consequence of variation in follicular development before the LH surge. In the

lactating sow, feed restriction affects the number of follicles in the preovulatory pool and their steroidogenic activity, as measured by the concentration of follicular fluid estradiol (Zak et al., 1997b). Therefore, as suggested by O'Callaghan et al. (1998), it seems likely that there will be carry-over effects on the pattern of luteinization of these follicles and their capacity for progesterone production after ovulation.

Short luteal phases in women and rhesus monkeys are associated with a decreased follicular phase FSH:LH ratio (Strott et al., 1970; Wilks et al., 1976). Similarly, Ramirez-Godinez et al. (1982) found a decrease in serum FSH prior to the first LH surge after weaning in cattle. These observations are consistent with the hypothesis that a relative deficiency of FSH during the follicular phase results in diminished follicular development and subsequent inadequate corpus luteum formation.

Characteristics of the preovulatory LH surge have been implicated in inadequate luteal function. Less et al. (1998) demonstrated that bovine granulosa cells undergoing luteinization *in vitro* produced more progesterone following exposure to a high LH 'surge' (15-100 ng/ml) than when they were exposed to lower doses of LH (0-10 ng/ml). *In vivo*, progesterone concentrations during the cycle were lower in cattle induced to ovulate with a low dose of Buserelin (2 μg) compared to the cattle administered higher doses of Buserelin (2 × 8 μg) or hCG (5000 IU) treatment. In the recent studies by Almeida et al. (2000b), feed restriction during the second week of the cycle in gilts affected the preovulatory LH surge, which would affect luteinization and contribute to the delayed rise in peripheral progesterone concentrations reported by Almeida et al. (2000b).

2.5. Hormone secretion during lactation and after weaning

Because the lactating and weaned sow was used to study the regulation of embryonic survival in two experiments reported in this thesis, it is important to understand neuro-endocrine control of ovarian function during lactation and after weaning. GnRH and gonadotropin secretion is predominantly inhibited by suckling of piglets. Feed restriction can further exaggerate this inhibitory effect on gonadotropin secretion. Thus, the impaired gonadotropin secretion may affect post-weaning fertility in the sow. The following section provides an understanding of the associations between

gonadotropin secretion and follicular development during lactation and reproductive performance after weaning.

2.5.1. Neuro-endocrine basis of lactational anestrus

Suckling is the oldest form of birth control, but it is only recently that we have understood the mechanisms involved in the suppression of follicular growth and development during lactation. Earlier reviews of the endocrinology of lactating sows have presented extensive evidence for a suckling-induced block of LH secretion as the principal cause of lactational anestrus (Britt et al., 1985; Varley and Foxcroft, 1990; Foxcroft, 1992; Quesnel and Prunier, 1995). However, this inhibitory control is absent in the immediate postpartum period, and only becomes well established by d 3-4 of lactation (Foxcroft, 1992). Then as lactation progresses, suckling intensity decreases, and suckling inhibitory effects on LH secretion gradually become less effective (Sesti and Britt, 1994).

A number of experimental approaches have shown that this inhibition of LH secretion results primarily from a block to the endogenous pulsatile release of GnRH at the level of hypothalamus (Foxcroft et al., 1992; Sesti and Britt, 1993a; Sesti and Britt, 1994). More convincing data to support the hypothesis that a lack of GnRH secretion is the primary cause of lactational anestrus came from studies using the pulsatile administration of GnRH at low dose during lactation. This regimen of GnRH treatment induces LH secretion (Sesti and Britt, 1994; De Rensis and Foxcroft, 1999) and is very effective in stimulating ovarian follicular development (De Rensis et al., 1991). If treatment persists, at least a proportion of sows show behavioral estrus and ovulate during lactation (Guthrie et al., 1978; Cox and Britt, 1982b; Rojanasthien et al., 1987). The number of medium and large follicles is also correlated with basal LH secretion during lactation (Sesti and Britt, 1993a; Sesti and Britt, 1994).

2.5.2. Prolactin secretion and the relationship with LH

Prolactin (PRL) secretion is responsible for the maintenance of lactation. Studies measuring PRL concentrations throughout lactation in the sow have shown that the concentrations are higher at the onset of lactation, when the demand for milk is high, than they are at late lactation. However, these concentrations at late lactation are still much higher than basal PRL concentrations seen during the estrous cycle (Edwards and

Foxcroft, 1983a). Compared with low PRL concentrations in "zero weaned" sows immediately after farrowing, PRL concentrations in suckled sows were higher at the same time (De Rensis, 1993; De Rensis et al., 1993b). PRL secretion decreased in response to transient weaning and increased again when the piglets were replaced (Armstrong et al., 1988a, b; Shaw, 1984). After weaning there is a dramatic decrease in circulating PRL concentrations (Edwards and Foxcroft, 1983b; Foxcroft et al., 1987; Rojkittikhun et al., 1991).

During lactation, there is some evidence for an increase in LH secretion during the periods of decreased PRL secretion (Shaw, 1984). Foxcroft et al. (1987) found that in lactation, LH was negatively correlated with PRL, and LH secretion robustly increased at the time of the dramatic decrease in PRL secretion shortly after weaning. LH secretion also increased and PRL secretion decreased during transient weaning during lactation (Armstrong et al., 1988a, b; Shaw, 1984), and in response to split-weaning (Grant, 1989). The inverse relationship between LH and PRL secretion during lactation and at weaning might suggest that high PRL concentrations during lactation mediate lactational anestrus. However, it is also possible that the neuroendocrine pathways involved in PRL secretion interact at the hypothalamic level to inhibit GnRH and thus LH secretion as discussed in the next section (reviewed by Kraeling and Barb, 1990). High prolactin concentrations may also have inhibitory effects on the ovaries (see Dusza and Tilton, 1990 for review).

Several studies have demonstrated that the endogenous opioid peptides are responsible for mediating the inverse relationship between LH and PRL secretion during lactation and at weaning. In the studies by Armstrong et al. (1988 a) and by De Rensis et al. (1993a), LH secretion increased and PRL secretion decreased, in response to treatment with the opiate antagonist, naloxone (NAL), during established lactation. These authors report that suckling bouts occurred during treatment with NAL, therefore, it appears that endogenous opioids released in response to suckling mediate the effects on these two hormones in lactation. In the lactating, ovariectomized (OVX) rat, naloxone infusion also increases LH release (Wu et al., 1992). Interestingly, De Rensis et al. (1993a) reported that the LH and PRL secretion in the sow is not affected by administration of NAL in the immediate post-partum period. In fact, there is no response until at least 78 hours post-partum, and weaning may not cause an immediate increase in LH and FSH, because LH

and FSH are not regulated through the opioid pathway (De Rensis et al., 1993a; Britt, 1996) at this stage. However, all these data suggest that opioids are involved in the regulation of LH suppression in established lactation in the sow.

2.5.3. GnRH, FSH and LH secretion and effects of litter size and feed intake

FSH and LH secreted by the anterior pituitary are under hypothalamic control via the release of GnRH. Suckling *per se* is the primary inhibitor of LH secretion during lactation and results in a reduced stimulation of the pituitary gland by GnRH. N-methyl-d, l-aspartate (NMDA), an analogue of the putative neurotransmitter glutamate/aspartate, can induce GnRH release. By challenging sows with NMDA, Sesti and Britt (1993a) have demonstrated that the releasable pools of GnRH increase as lactation progresses, and *in vitro* studies have also shown that the rate of GnRH release increases as lactation proceeds (Sesti and Britt, 1993b).

There are fewer data concerning the variation in FSH secretion during lactation. Within the first 3 d after parturition, FSH concentrations do not vary with time and are similar in suckled and zero-weaned sows (De Rensis et al., 1993b). In both types of females, high variability is observed between individuals. From the second week of lactation onwards, a continuous increase in plasma FSH has been observed by Stevenson et al. (1981) and De Rensis et al. (1993b). In comparison, for at least the first 3 days after farrowing LH secretion is still very pulsatile in the sow, resembling that of follicular phase animals; however, by d 3 of lactation, LH secretion is significantly suppressed by suckling (Tokach et al., 1992a; De Rensis et al., 1993b). Studies in lactating sows and sows weaned immediately after farrowing (zero-weaned) shows that although mean plasma concentrations and pulsatility of LH are similar in both groups at d 1, there is a difference in LH secretion as early as d 4 (Smith et al., 1992; De Rensis et al., 1993b), and suckled sows move from a 'hypergonadotropic' to a 'hypogonadotropic' phase of lactational anestrus (Britt, 1996; Figure 2.2). Depending on the metabolic state of the sow, pulsatile LH secretion may then gradually increase as lactation progresses (Stevenson et al., 1981; Koketsu et al., 1996; van den Brand et al., 2000a).

The study of Stevenson et al. (1981) in sows ovariectomized during lactation clearly demonstrated that LH and FSH secretion is differentially regulated in lactation and an inverse relationship between plasma LH and FSH concentrations was

subsequently reported in the lactating (Foxcroft et al., 1987; Mullan et al., 1991) and in the weaned sow (Trout et al., 1992). This is consistent with the concept that an increase in episodic LH secretion promotes follicular development, which in turn increases inhibin production, and thereby produces a relative decrease in circulating FSH by feedback of inhibin.

Effects of litter size: A reduction in litter size during lactation (from 10 to 5 piglets) is not apparently associated with any persistent proportional reduction in plasma prolactin concentrations (Shaw, 1984; Grant, 1989), suggesting that the afferent input from a limited number of piglets is sufficient to exceed the threshold for blocking the dopaminergic inhibition of prolactin secretion. Complete separation of the litter, however, on either a temporary or permanent basis, results in a precipitous decline in prolactin secretion to basal levels within 6 h (Shaw, 1984). Consistent with reports of a beneficial effect of fractionated or split-weaning on reproductive function (Britt et al., 1985), Shaw (1984) observed that a reduction in litter size on d 21 of lactation resulted in a marginal stimulation of follicular development determined *in vitro* on d 28. However, there was no evidence of a persistent change in gonadotropin secretion in the 24-h period before final weaning at d 28 and only a transient increase in FSH, and no effect on LH, in the 12-h period after initial litter size reduction.

Reducing the intensity of the piglet suckling stimulus, by removal of the heaviest piglets (split weaning) decreases the weaning-to-estrus interval (Stevenson and Britt, 1981). To determine whether reduced suckling *per se* or a reduced metabolic demand of milk production, result in enhancement of follicular development after split weaning, Grant (1989) removed the 5 largest piglets from each litter at d 14 of lactation and allowed the remaining piglets either to suckle all the available teats or only six (covered). Although total litter weight were similar among all treatments, follicular development and aromatase activity was greater in the covered group. LH increased transiently after split weaning, although no consistent relationship between ovarian development and LH was observed. From this experiment it is apparent that the greatest ovarian development was observed in the covered sows, and although the litter weight gains were similar between the covered and split weaned groups, the net suckling intensity in the covered group was reduced.

Effects of nutritional state: There is extensive evidence for nutritional effects on LH secretion during lactation. Quesnel et al. (1998) and Zak et al. (1998) compared the effects of *ad libitum* and 50% of *ad libitum* feed intake (feed restriction) throughout 28-d lactation and suckling by 8 to 10 piglets on LH secretion in primiparous sows. Feed restriction in these experiments suppressed LH secretion (both LH mean concentrations and pulse frequency), consistent with the concept that a negative energy balance in lactation will trigger changes in circulating metabolites and metabolic hormones that have the potential to inhibit GnRH release independently of suckling-induced inhibition of LH secretion (Foxcroft, 1992). Similar results were reported by Mullan et al. (1991). In another experiment, in which the number of suckling piglets was reduced to 6, Zak et al. (1997 a) examined the effect of different patterns of feed restriction on LH secretion in primiparous sows. Three patterns of feeding were studied: AA sows were fed to appetite (100%) throughout a 28-d lactation period. AR sows were fed to appetite from farrowing to d 21 and then restrict fed to 50% of to appetite feed intake from d 22 to 28. RA sows were restrict fed from farrowing to d 21 and then fed to appetite from d 22 to 28. Notwithstanding the dominant inhibitory effect of suckling, feed restriction again completely suppressed episodic LH secretion during lactation and differences in the patterns of feed intake produced significant effects on post-weaning fertility.

Comparing litter size and feed restriction, inhibitory effects of suckling on the LH secretion appear to be predominant over feed restriction. Mullan et al. (1991) compared the effects of two levels of energy intake during lactation, and suckling by either 6 or 12 piglets, on LH secretion. Plasma LH secretion was consistently less in sows being suckled by 12 as opposed to six piglets. Although feed restriction can exaggerate this inhibitory effect of suckling (Quesnel et al., 1998; Zak et al., 1997a, 1998), making sows anabolic during lactation by superalimentating to 125% of *ad libitum* feed intake did not abrogate the suckling-induced inhibition of LH secretion (Zak et al., 1998).

2.5.4. Endocrinology of the sow at weaning

Weaning results in dramatic changes in hormone secretion. Although the FSH response to weaning can be variable, there are indications that FSH concentrations generally increase gradually after weaning (Edwards and Foxcroft, 1983a; Shaw and Foxcroft, 1985; Foxcroft et al., 1987) and that the increase in FSH is concomitant with

follicular growth and maturation (Britt et al., 1985). In contrast, basal plasma LH concentrations and frequency of episodic pulses of LH increase significantly from the day before weaning to higher levels on the day after weaning (Cox and Britt, 1982a; Edwards and Foxcroft, 1983a, b; Shaw and Foxcroft, 1985; Foxcroft et al., 1987; Zak et al., 1997a, 1998; Quesnel et al., 1998; van den Brand et al., 2000a). The increase in LH secretion is associated with similar increases in hypothalamic GnRH content (Cox and Britt, 1982b) within 60 h of weaning, thereby increasing the potential for LH synthesis by the pituitary and for a sustained LH secretion at estrus. It has been suggested that this increase in the magnitude and pulse frequency of LH is functionally related to the rate of follicular growth and hence to the weaning-to-estrus interval (Shaw and Foxcroft, 1985; van den Brand et al., 2000a).

2.5.5. Weaning-to-estrus interval (WEI)

WEI, the interval from the time of weaning to first detection of standing heat, is an important part of the normal reproductive cycle of the sow. Generally speaking, a high weight loss during lactation, as induced by feed restriction, or reduced voluntary feed intake, resulted in an increase in WEI especially for the primiparous sows (den Hartog et al., 1994; Koketsu et al., 1996). A number of factors that affect energy balance and body weight change will influence WEI. These factors include lactation length (Xue et al., 1993), breed (Vesseur, 1997), litter size at weaning (Vesseur, 1997), season (Vesseur, 1997), feeding levels and regimen during lactation (Zak et al., 1997a; 1998; van den Brand et al., 2000b) and feeding level after weaning (King and Willians, 1984a, b). Dietary protein concentration during lactation did not have any effects on WEI (Johnston et al., 1993).

A delay in estrus after weaning may result in subsequent poor reproductive performance. It is reported that sows inseminated at d 4 or 5 after weaning had more piglets born alive after first insemination than sows inseminated at d 8 to 12 (den Hartog et al., 1994). An increase in WEI results in a decrease in the duration of estrus and a decrease in the interval from onset of estrus to ovulation (Rojkittikhun et al., 1991; Kemp and Soede, 1996). This can have consequences for the time of insemination during estrus. The sows that come into heat later should be inseminated earlier, compared with the sows that have shorter WEI, otherwise, fertilization rate will be affected.

So far, follicular development and its regulation, GnRH, FSH and LH secretion during lactation, and their significance for swine reproductive performance after weaning have been discussed. Feed restriction, as discussed above, affects metabolic and gonadotropin hormone secretion, follicular development and embryonic survival. Insulin and leptin have been studied in different metabolic states, especially in feed restricted animals, and are suggested to be good indicators of metabolic states. These hormones may also be the link between nutrition and reproduction. Our understanding of how these hormones link metabolic states by acting at the level of the hypothalamus and pituitary to affect gonadotropin secretion centrally will then be discussed in the last section of this review.

2.6. Metabolic cues and LH secretion

Energy balance, the net difference between energy intake and energy expenditure, has a profound impact on reproduction (Bronson, 1987). Acute alterations in nutritional status have profound effects on reproductive function, for example in the gilt (Booth et al., 1994), lactating sow (Koketsu et al., 1996; Zak et al., 1997a) and monkey (Cameron and Nosbisch, 1991). A common feature of all these studies is that disruption of reproductive processes is associated with the suppression of pulsatile LH release. Intermittent infusion of GnRH to feed restricted monkeys (Dubey et al., 1986), and challenges with NMDA in the growth restricted lamb (Landefeld et al., 1989), both restored the pulsatile release of LH. These data suggest that nutritional modulation of LH secretion appears to be affected at the level of the GnRH pulse generator. Recent studies demonstrated that undernutrition-induced inhibition of LH secretion involves both an indirect suppression of LH secretion via amplification of endogenous steroid negative feedback (Dong et al., 1994), as well as direct suppression of GnRH release (I'Anson et al., 2000), but did not involve a change in LH clearance rate (Dong et al., 1993).

The mechanisms for suppression of pulsatile GnRH release are still not well understood and many hypothalamic neuromodulators have been invoked as potential central regulators of GnRH release. NPY plays a key role in the nutritional inhibition of GnRH, since gene expression for NPY is increased in most forms of 'metabolic hypogonadism' in rats, and Pierroz et al. (1996) demonstrated that exogenous NPY could reproduce hypogonadism. The discovery of leptin and its role as a regulator of feed

intake, the role of NPY as a possible neurotransmitter mediating the hypothalamic actions of leptin, and the significant observations that leptin could maintain reproductive function in fasting animals, has contributed to our understanding of the link between nutrition and reproduction. As an integral part of nutrition-reproduction interactions, the link between nutritional deprivation and inhibition of GnRH secretion has been extensively studied in a number of species (I'Anson et al., 1991). Nutrient availability was suggested as one part of the link.

2.6.1. Nutrient availability

Nutrient availability has a great effect on LH secretion. In the fasted monkey, reinstatement of LH pulsatility is dependent on the size of the meal (the energy value) at realimentation (Parfitt et al., 1991). The reinstatement of LH secretion is not due to stomach distension as a result of the mass of feed, since saline infusion into the stomach of fasted monkeys did not increase LH, whereas gastric infusion of feed did (Schreihofer et al., 1993). Subsequently, Schreihofer et al. (1996) found that the reinstatement of LH pulsatility after a 48 h fast was not dependent on the source of nutrients ingested, and feeding of macro-nutrient diets containing pure fat, protein or carbohydrate all increased LH secretion. The identification of the metabolic signals which mediate such changes in LH, and presumably GnRH neuronal activity, are therefore of great interest. In the gilt, ingestion of sugar beet fibre, which has a low energy density, did not increase LH pulsatility in contrast to the normal high energy diet (Formigoni et al., 1996).

Many putative metabolic signals have been proposed to link nutrition and somatic metabolism with reproductive function, including gut peptides and other metabolic hormones (Foxcroft, 1990; I'Anson et al., 1991; Miller et al., 1995). The concentrations of metabolic fuels (glucose, amino acids and free fatty acids) and metabolic hormones (insulin, IGF-I, GH, prolactin etc) in the cerebrospinal fluid of adult male sheep affected by different feeding levels were studied by Miller et al. (1998). In that study, adult sheep were fed either maintenance or 2.5 times maintenance energy requirements. Concomitant with the increase in LH and FSH secretion, there was an increase in cerebrospinal fluid concentrations of insulin, glucose and certain amino acids such as phosphoserine, glutamine, GABA (γ-aminobutyric acid), threonine, tyrosine and phenylalanine. Since

phenylalanine, tyrosine and trypophan are also the precursors for catecholamines and indoleamines, it was suggested that insulin, glucose and these amino acids may be important links in the mechanism by which nutrition influences reproduction (Foxcroft, 1990; Miller et al., 1998). Pharmacological reduction of glucose availability with 2-deoxyglucose (2-DG) interrupts estrous cyclicity in the Syrian hamster (Schneider et al., 1993) and suppresses pulsatile LH secretion in lambs (Bucholtz et al., 1996). Wade et al. (1996) accumulated a vast body of evidence to suggest that glucose availability in hamster, rats, sheep, cattle, and rhesus monkey is one of the regulators of LH secretion.

Two approaches have been employed to investigate the role of glucose in regulating LH secretion. One is hypoglycemia induced by high doses of insulin. Depletion of glucose by insulin-induced hypoglycemia in the rhesus monkey (Chen et al., 1996), sheep (Clark et al., 1990; Adam and Findlay, 1998; Medina et al., 1998) and rats (Rodriguez et al., 1999) inhibits the activity of the GnRH pulse generator and reduces LH release. Intrahypothalamic or intravenous glucose infusion restores LH secretion to normal (pulse frequency, maximum and basal secretion) in rats and sheep. The second approach is glucoprivation induced by 2-DG, a competitive antagonist of glucose metabolism. Intracerebroventricular or systemic administration of 2-DG suppresses LH secretion in rats (Bucholtz et al., 1996; Briski, 1997; Tsukahara et al., 1999), independent of the peripheral glucose concentration.

The undernourished, gonadectomized, lamb model was also developed in Foster's group. Since there is little body fat to buffer nutritionally-mediated alterations in metabolic status, the GnRH neuronal system is exquisitely sensitive to acute changes in nutrient availability. A characteristic of this model is an increase in peripheral glucose upon refeeding, associated with an acute increase in LH pulsatility (Bucholtz et al., 1993). It can therefore be postulated that abrupt changes in glucose availability mediate abrupt diet-induced changes in LH secretion.

Murahashi et al. (1996) provided histological evidence showing that glucose availability could influence LH secretion through a central sensor in the lower brain stem, consistent with the idea that the *area postrema* might be an important glucosensor involved in the modulation of LH secretion. The insulin sensitive glucose receptor, GLUT4, has been identified in the hypothalamus of the rat, and may mediate a functional

route by which glucose could modulate the neuronal activity of GnRH either directly or indirectly (Livingstone et al., 1995).

In some models, however, other factors are involved and the experimental results do not support a role for glucose availability on LH secretion. For example, the glucoprivic suppression of LH pulses is potentiated by gonadal steroids (Nagatani et al., 1996; Briski, 1997). LH secretion in gonad-intact females and estrogen-treated OVX monkeys appears to be more sensitive to blood glucose levels (Chen et al., 1992). In the OVX monkeys, insulin-induced hypoglycemia suppresses LH secretion only when glucose levels fall to less that 40% of basal concentrations, but in estrogen-treated and intact monkeys, a decrease in plasma glucose of only 10-15% was capable of suppressing LH secretion in some animals. In the pig, glucose infusion in primiparous feed restricted lactating sows, which are suckling 9 piglets, did not increase LH pulsatility (Tokach et al., 1992b), possibly because of the overriding intensive inhibition from suckling stimulus on LH secretion or possibly because the model used did not allow marginal effects on episodic LH secretion to be established. However, in prepubertal gilts in which 7 days of feed restriction has blocked LH pulsatility, glucose injections were as effective as realimentation in triggering an immediate increase in LH secretion (Booth, 1990).

2.6.2. Insulin

Endogenous insulin is normally secreted in response to circulating nutrients as well as in proportion to the degree of adiposity. Thus, insulin concentrations in blood are a reliable indicator of adiposity. The role of insulin in the adaptive response of peripheral tissues to changes in energy balance is well-established (Schwartz et al., 1992a). This central position in the adaptive response of peripheral tissues makes insulin a logical candidate for an afferent central nervous system (CNS) signal, transducing changes in metabolic status to the reproductive axis. Several lines of evidence from the rat and monkey confirm that insulin receptors are widely distributed in the brain, with highest concentrations in the membranes from cerebral cortex and hypothalamus (Marks and Eastman, 1990). In the hypothalamus, insulin receptor mRNA was found to be concentrated in neurons of the arcuate nucleus in the rat (Marks and Eastman, 1990). In the rat, peripheral insulin is positively transported into the CNS by means of a saturable transport system (Schwartz et al., 1990), the same transport mechanism as leptin. In the

rhesus monkey, intragastric infusion of casein and lipids, which does not restore glucose concentrations but does restore insulin concentrations, is able to re-initiate pulsatile LH secretion (Schreihofer et al., 1996).

I.c.v. administration of insulin into ovariectomized gilts increased LH frequency (Cox et al., 1989) and *in vitro* incubation of medial basal hypothalamic fragments with insulin and glucose in the rat potentiates the release of GnRH when compared to glucose alone (Arias et al., 1992). Studies in the mature ram which were feed restricted and then given the equivalent energy supplementation of lupin feeding, in the form of intracerebroventricular (*i.c.v.*) glucose and insulin, or insulin alone for 12 h/day for 4 days, also increased LH pulsatile release (Miller et al., 1995). Similarly, in the prepubertal gilt additional energy supplied to maintenance fed gilts in the form of a glucose infusion caused an immediate increase in plasma insulin that may have mediated the observed immediate increase in LH frequency (Booth, 1990). In lactating sows, it was found that the mean concentration of plasma insulin as early as d 7 postpartum was positively correlated with the number of LH peaks observed at d 14 and 21 postpartum (Tokach et al., 1992 a). However, in another study of Tokach et al. (1992 b), although infusing glucose on d 18 postpartum for 12 h increased insulin concentrations, mean LH concentration and LH pulsatility did not change. Similarly, although peripheral infusion of glucose for 4 h/d for 10 days, to mimic the energy content of lupin supplemental feeding in the mature ram, increased peripheral insulin and glucose concentration, it did not affect LH secretion (Boukhliq et al., 1996). Furthermore, Hileman et al. (1993) injected insulin *i.c.v.* into growth restricted ovariectomized lambs and did not observe an increase in LH. Williams et al. (1996) could not establish an absolute role of insulin in the increase of LH secretion in response to realimentation in the monkey either. In conclusion, insulin alone does not seem to be able to signal metabolic state to the reproductive system. However, it may interact with other factors, such as leptin and NPY, to regulate hypothalamic function.

2.6.3. Leptin

The *ob* gene encodes a 16-kd protein, called leptin, that is synthesized mainly in adipose tissue in pigs (Mendiola et al., 1997) and is considered to be a modulator of feeding behavior in mice (Campfield et al., 1995). At the amino acid level, porcine leptin

has 86% homology to human leptin and 93% homology to bovine leptin (Mendiola et al., 1997). It was of comparatively little interest for most researchers that ob/ob (absence of the leptin protein) or db/db (absence of leptin receptors) mutant mice were basically infertile until Chehab et al. (1996) found that leptin injections into adult ob/ob mice restored their reproductive capacity. In that experiment, the ob/ob mutant female mouse, which does not produce an active form of leptin due to a mutation of the ob gene, was shown to be acyclic and sterile, whether they were induced to lose weight by diet restriction or not. However, this sterility could be reversed by treatment with recombinant leptin, suggesting that leptin is required for normal reproductive function. In another experiment, administration of leptin in these animals increased basal LH secretion (Baras et al., 1996). Based on these data, it has been suggested that leptin serves as a metabolic signal to the reproductive system and several lines of evidence support this hypothesis.

The levels of leptin are elevated in obese states such as feeding carbohydrate (Thompson, 1996) and low in states of starvation (Frederich et al., 1995). Significantly, a saturable unidirectional transport system across the blood-brain barrier has been identified, facilitating leptin entry into the CNS at a rate similar to other blood-borne regulatory compounds (Banks et al., 1996).

Another important discovery is that the leptin receptor mRNA transcript is highly expressed in hypothalamic GnRH neurons (Lee et al., 1996; Zamorano et al., 1997), suggesting that leptin can act on GnRH neurons to regulate LH secretion. The ob/ob mouse reportedly has decreased plasma gonadotropin levels (Swerdloff et al., 1976) and treatment with exogenous leptin in these mice enhances both plasma luteinizing hormone secretion (Ahima et al., 1996; Barash et al., 1996) and increases follicular maturation (Barash et al., 1996). Furthermore, fasting in rodents and humans decreased plasma leptin concentrations (Maffei et al., 1995; Kalra et al., 1998) and also depressed LH secretion, and leptin administration i.p. or *i.c.v.* restored LH secretion to the level of fed animals (Nagatani et al., 1998; Kalra et al., 1998). Finally, in an experiment designed to mimic the fall of leptin levels during fasting, leptin antibody was injected into the rat lateral ventricle, and caused a decrease in LH pulsatility and a cessation of estrous cyclicity (Carro et al., 1997). Taken together, available data support the concept that leptin plays an important role in regulating the hypothalamic-pituitary axis.

Immunohistochemical studies revealed dense immunolocalization of the leptin receptor in the choroid plexus, and in the arcuate nucleus/median eminence of the female rat, a key site in the control of feeding and reproduction (Zamorano et al., 1997). It was also demonstrated that leptin receptor mRNA is highly concentrated in the arcuate nucleus and that *i.c.v.* administration of leptin reduces levels of NPY mRNA in this hypothalamic area (Schwartz et al., 1996b), suggesting that the arcuate nucleus is a likely target of leptin action. The concept that leptin acts on multiple hypothalamic targets is consistent with the proposed role of this hormone as an important physiological signal in the control of energy balance. In addition, high-affinity leptin binding has been found in the hypothalamus (Stephens et al., 1995), as well as other areas such as the choroid plexus (Tartaglia et al., 1995).

Since fasting markedly decreases leptin production and release from adipocytes (Ahima et al., 1996; Maffei et al., 1995), it is likely that leptin reaching the brain is, likewise, drastically reduced (Banks et al., 1996). The ob/ob genotype of mouse is characterized by having elevated plasma level of NPY (Schwartz et al., 1996a). In feed-deprived rats, hypothalamic prepro-NPY mRNA levels also increase significantly, concomitant with suppression in serum LH levels (Kalra et al., 1998). Leptin treatment decreases the levels of mRNA for NPY in the hypothalamic arcuate nucleus in both ob/ob mice (Stephens et al., 1995; Schwartz et al., 1996a) and feed-deprived rats (Kalra et al., 1998), which is the principle location of NPY cell bodies (Bai et al., 1985). These observations imply that the hypothalamic NPY system is likely to be one of the central loci of leptin action and that over-expression in the hypothalamic NPY system may contribute to marked suppression in gonadotropin secretion (see section 2.6.4 for discussion). Further, the finding that leptin receptor mRNA and NPY mRNA are co-localized in the arcuate nucleus (ARC) (Mercer, 1996), clearly indicates that NPY perikarya are the site where leptin acts to down regulate NPY gene expression, and thereby diminish the supply of NPY in the terminal fields in the paraventricular nucleus and surrounding sites. Continuous activation of NPY receptors in these sites results in decreased gonadotropin release in feed-deprived animals, whereas a leptin-induced decrease in NPY supply at these sites underlies the recovery of gonadotropin secretion (Kalra and Kalra, 1996; Kalra et al., 1998). It has been showed that central infusion of

NPY into the lateral ventricle of normal rats rapidly inhibits gonadotropin secretion (Catzeflis et al., 1993; Pierroz, 1996).

On the other hand, the concentrations of leptin are regulated by several other hormones, such as glucocorticoids (de Vos et al., 1995) and insulin (Saladin et al., 1995; Kolaczynski et al., 1996). There is a significant relationship between insulin and leptin, but it is not immediate, since leptin is produced only as a response to the insulin stimulus under conditions of high energy availability (Saladin et al., 1995; Kolaczynski et al., 1996). Insulin alone, without the concurrence of high glucose levels, fails to raise leptin concentrations. Interestingly, studies from Schneider's group on the Syrian hamster showed that the positive action of leptin on estrous cyclicity during fasting is inhibited by 2-DG treatment (Schneider et al., 1998; Schneider and Zhou, 1999), suggesting that glucose, insulin, leptin, NPY and other factors form an intricate system to regulate nutritional effects on LH secretion.

2.6.4. Neuropeptide Y

Neuropeptide Y (NPY) is a 36-amino-acid peptide that belongs to the family of pancreatic polypeptides. High levels of NPY are detected in the central and peripheral nervous system in human and animals (Heilig and Widerlov, 1995). NPY functions as a neurotransmitter or neuromodulator (Heilig and Widerlov, 1995), and its action is mediated by specific receptors, Y1, Y2, Y3, Y4, Y5, and Y6 (Balasubramaniam, 1997). Recent experimental evidence indicates that genetic, hormonal, and environmental factors that cause nutritional imbalance and metabolic disturbances, along with depressed pituitary function, produce alterations in the synthesis, storage, and release of NPY in the hypothalamus. Therefore, NPY may be one of the essential messenger molecules that serve as a communication bridge between the neural processes that regulate reproduction and those that maintain energy homeostasis (Kalra and Kalra, 1996). NPY has been shown to regulate the activity of the reproductive axis. NPY can stimulate LH release in steroid-primed OVX rats after central administration of NPY. In contrast, in castrated animals, central administration of NPY produced a striking inhibition of LH release in rats (Kalra and Crowley, 1984; McDonald et al., 1989) and in rhesus monkeys (Kaynard et al., 1990). The conflicting effects of NPY on GnRH secretion at the hypothalamic level are mediated via different receptor subtypes. Recent studies showed that NPY Y1 and Y4

mainly mediate stimulatory responses (Kalra et al., 1992; Jain et al., 1999), while NPY Y5 mediates inhibitory responses (Raposinho et al., 1999). The excitatory and inhibitory effects of NPY on GnRH and LH secretion are also dependent on the steroidal milieu (Urban et al., 1996). Finally, Vanhatalo and Soinila (1996) showed that NPY-containing neuron project to the pituitary, indicating that NPY may also affect pituitary functions by affecting blood flow.

2.6.4.1. Excitatory effects of NPY on LH secretion

Two modalities of hypothalamic GnRH secretion have been identified: a basal and a cyclic mode. A number of studies show that in the rat NPY is excitatory to both the episodic basal release of LH, and to the preovulatory cyclic LH surges (Kalra, 1993). The basal mode consists of low amplitude bursts of GnRH secretion at frequencies that vary during different phases of the estrous cycle in the female, and NPY is secreted in discrete episodes in the basal hypothalamus which coincide temporally with GnRH pulses (Woller et al., 1992). NPY Y4 receptors are thought to play a role in this stimulation of basal LH release (Jain et al., 1999). During the proestrous period, an accelerated rate of GnRH secretion is superimposed on this low basal secretion. This cyclic burst of hypothalamic neurosecretion induces a massive preovulatory discharge of gonadotropins which cause ovulation (Kalra and Kalra, 1983; Kalra, 1986). The hypothalamic NPY system is closely associated with this cyclic discharge of LH; NPY levels in the median eminence, secretion of NPY from the median eminence (Watanabe and Takebe, 1992), and NPY mRNA expression in the ARC increased before and during a spontaneously LH surge at proestrus, or during a surge induced by progesterone in estrogen-primed OVX rats (Sahu et al., 1994, 1995). The lack of an increase in median eminence (ME) NPY and NPY mRNA expression attenuated LH secretion in middle-aged rats, compared to the increased NPY in ME before and during the LH surge in young rats (Sahu and Kalra, 1998). Blockade of NPY synthesis by antisense oligodeoxynucleotides inhibited NPY accumulation in the median eminence and blocked the progesterone-induced LH surge in OVX rats (Kalra et al., 1995). Similarly, blockade of the effects of NPY by passive immunoneutralization inhibited the LH surge induced by progesterone and that occurring spontaneously at pre-estrus (Wehrenberg et al., 1989; Sutton et al., 1988) and also LH pulsatility in the OVX rats (Xu et al., 1993, 1996). Intraventricular administration of

NPY receptor antagonist also blocked the progesterone-induced LH surge in OVX rats (Jain et al., 1999). Furthermore, intraventricular administration of NPY stimulated LH release in OVX rats primed with steroids (Kalra and Crowley, 1992). Studies also show that gonadal steroids are necessary to maintain GnRH responsiveness to NPY receptor stimulation (Besecke and Levine, 1994; Urban et al., 1996). It was shown that NPY effectively stimulates GnRH release from ME fragments *in vitro* only when OVX rats had been treated with estradiol and high doses of estradiol elicit a greater GnRH secretory responses to NPY (Sabatino et al., 1989 and 1990). Steroids may thus regulate GnRH responsiveness to NPY so as to permit information regarding the nutritional status of the animal to impact reproductive status. The medial preoptic area (MPOA) is one of the sites of the excitatory action of NPY. Several lines of evidence have proved that the NPY network in the ARC-ME-MPOA axis represents an integral link in the neural circuitry engaged in excitation of the two modalities of GnRH secretion (Kalra and Kalra, 1996).

In addition to such central effects on the release of GnRH, NPY also acts at the level of pituitary gonadotropins, where it amplifies the GnRH-induced release of LH (Crowley and Kalra, 1988). After release into the hypophysial vasculature from nerve terminals in the ME, NPY potentiates GnRH-induced LH release from the pituitary and this effect is steroid-dependent (Kalra and Crowley, 1992). This unique ability to potentiate the effectiveness of GnRH may be responsible for quantitatively increasing the magnitude of the preovulatory LH surge (Kalra and Crowley, 1992). It may also contribute to maintenance of LH secretion when GnRH secretion is either diminished, or markedly suppressed, in response to environmental challenges such as nutritional deficiency or undernourishment (Schreihofer et al., 1993; Dong et al., 1994). Peripheral administration of the NPY receptor antagonist attenuated the LH hypersecretion induced by GnRH and NPY analogs in pre-estrous rats (Leupen et al., 1997), supporting the concept that pituitary NPY receptors may mediate the effects of exogenous NPY on gonadotropin secretion.

The precise anatomical localization of NPY receptors mediating actions on the reproductive system has not been identified, although binding sites for NPY are found in a number of nuclei within the hypothalamus, including the MPOA, ME and ARC (Dumon et al., 1993). Although there is no direct evidence to indicate that GnRH neurons

contain NPY receptors, it has been demonstrated that NPY terminals are juxtaposed with GnRH neurons (Calka et al., 1993) and that NPY directly, and dose dependently, stimulates GnRH release (Besecke et al., 1994).

2.6.4.2. Inhibitory effects of NPY on LH secretion

Excitation of GnRH-LH release requires intermittent hypothalamic NPY receptor activation. However, continuous receptor activation, even in steroid-primed ovariectomized rats, inhibits LH release instead of exerting an excitatory influence (Kalra, 1986). It has been reported that continuous intraventricular infusion of NPY for 7 days inhibits pituitary-gonadal function in male and female rats (Pierroz et al., 1995), with a collapse of estrous cyclicity (Catzeflis et al., 1993) and delayed sexual maturation in female rats (Pierroz et al., 1995). In castrated animals, central administration of NPY produced acute inhibition on LH release (Raposinho et a., 1999). Episodic GnRH discharge is vital for normal pituitary-gonadal function (Kalra, 1993) and it is dependent upon the intermittent action of NPY (Woller et al., 1992; Xu et al., 1993). Thus, any derangement in the NPY secretion pattern in the hypothalamus results in reduced pituitary-gonadal function. It has been demonstrated that in both the absence and the presence of extremely low levels of gonadal steroids, NPY injections invariably suppress LH secretion (Kalra, 1986; McShane et al., 1992; Pierroz et al., 1995). 'Thus it is clear that a derangement in the pattern of NPY receptor activation along with diminished gonadal steroids causes a loss in the excitatory effects of NPY on GnRH secretion. These results suggest that NPY is involved in the central mediation of nutrition-reproduction interactions' (Kalra and Kalra, 1996).

2.6.4.3. Effects of food restriction on NPY and LH secretion

Food restriction causes a reduction in LH secretion. Interestingly, this disruption of LH secretion in food-restricted animals is associated with altered hypothalamic NPY output. Several studies have shown that food restriction increases concentrations of NPY and prepro NPY mRNA expression in the central nervous system in the sheep (McShane et al., 1992; 1993), rat (Beck et al., 1992; Brady et al., 1992) and Jungarian hamster (Mercer et al., 1995). Increased hypothalamic preproNPY mRNA and elevated NPY levels in the hypothalamic sites (McShane et al., 1993) and cerebrospinal fluid (McShane

et al., 1992) induced by feed restriction were associated with suppression of LH release in the sheep and rat (Kalra et al., 1998). Moreover, peripheral or central administration of NPY to rats (McDonald, 1988) and sheep (Miner et al., 1989) induces feeding, and attenuates LH secretion in OVX rats (Kalra and Crowley, 1984; McDonald et a., 1988), rabbits (Khorram et al., 1987) and sheep (McShane et al., 1992). Therefore, during the restriction period, continuous activation of hypothalamic NPY receptors stimulates feeding but decreases LH secretion in gonadectomized and intact sheep (Ober et al., 1992), and restriction-induced up-regulation of the hypothalamic NPY system may attenuate the excitatory effects of NPY on GnRH secretion. This inhibition of GnRH secretion is shown to delay sexual maturation in rats (Pierroz et al., 1995).

2.6.4.4. Regulation of NPY by leptin and insulin

The stimulation of hypothalamic NPY biosynthesis during starvation and feeding is very sensitive to the circulating levels of insulin and leptin. In insulin-deficient states, such as diabetes mellitus, hypothalamic expression of preproNPY mRNA is augmented. Studies in both the spontaneously diabetic BB rat (Abe et al., 1991), as well as in streptozotocin-induced diabetic animals (Sahu et al., 1990), revealed increased hypothalamic levels of both NPY and its mRNA, and both could be normalized with systemic insulin therapy. Systemic administration of insulin (Schwartz et al., 1992b) and leptin (Stephens et al., 1995; Schwartz et al., 1995; Schwartz, 1996a,b; Ahima et al., 1996; Ahima et al., 1999) inhibit the increase in NPY gene expression in the arcuate nucleus associated with fasting (Strack et al., 1995). Leptin also inhibits glucocorticoid-stimulated hypothalamic NPY release *in vitro* (Stephens et al., 1995). The demonstration of receptors for insulin (Schwartz et al., 1992a) and leptin (Zamorano et al., 1997) on arcuate nucleus neurons is consistent with these regulatory actions. Thus, during fasting or feed restriction, activation of the hypothalamic NPY pathway is mediated, at least in part, by reduced concentrations of leptin and possibly insulin (Schwartz and Seeley, 1997; Figure 2.4). Elevated NPY activity evokes feeding, depresses GnRH secretion, and inhibits reproductive function. Evidence for this model is derived from the ob/ob, NPY knockout mouse, in which deletion of NPY not only attenuates the obesity syndrome but also improves reproductive and other neuroendocrine disorders characteristic of leptin deficiency (Erickson et al., 1996).

Based on the above work that has been carried out in rodent, hamster, monkey, sheep and even human, it can be concluded that insulin, leptin, and NPY participate in the basic system which signals metabolic status and regulates LH secretion in the long-term, while abrupt changes in glucose availability may mediate short-term changes in LH secretion.

2.7. Conclusions

There is convincing evidence to show that nutrition and metabolic state can affect gonadotropin secretion, follicular development, luteal function and subsequent embryonic survival in pigs. Asynchrony between the uterus and embryo and within-litter diversity may be key factors determining embryonic loss in the pig, and may in part come from follicular heterogeneity. Thus factors such as gonadotropins, metabolic hormones and growth factors that regulate follicular growth and oocyte maturation before ovulation, and are themselves influenced by nutritional status, may indirectly affect early embryonic survival. A critical link between nutritional effects on follicular maturation and the uterine environment, in which embryonic development takes place, appears to be progesterone status in the immediate post-ovulatory period. Direct evidence to support this suggestion is available from studies in cyclic gilts but not in lactating and weaned sows; the first experiment presented in Chapter 3 of this thesis addressed this deficiency. In Chapter 5, nutritionally-induced effects on early luteal function were explored in an established cyclic gilt model, as another approach to further elucidating progesterone-mediated effects of nutrition on fertility in the pig. The earlier parts of this literature review indicate the physiological mechanisms by which previous nutrition might affect subsequent luteal function.

GnRH secretion plays a central role in the reproduction process. However, there are only a handful of recent studies on the regulation of gonadotropin secretion by nutrition and subsequent effects on reproductive performance available in the pig. In the study reported in Chapter 4 in thesis, a second study in lactating and weaned sows was designed to provide evidence that differences in pulsatile LH secretion during lactation critically influence fertility after weaning.

The information provided in the later parts of this literature review, derived largely from rodent studies, provide evidence for some of the key central mechanisms

that may mediate central nutritional effects on the reproductive system. This information helps to suggest further approaches that may be taken to investigate similar mechanisms in future studies in lactating and weaned sows.

LITERATURE CITED

Abe M, Saito M, Ikeda H, Shimazu T. Increased neuropeptide Y content in the arcuate-paraventricular hypothalamic neuronal system in both insulin-dependent and non-insulin dependent diabetic rats. Brain Res 1991; 539: 223-227.

Adam CL, Findlay PA. Inhibition of luteinizing hormone secretion and expression of c-fos and corticotrophin-releasing factor genes in the paraventricular nucleus during insulin-induced hypoglycaemia in sheep. J Neuroendocrinol 1998; 10: 777-783.

Adashi EY. Regulation of intrafollicular IGFBPs: possible relevance to ovarian follicular selection. In: Baxter RC, Gluckman PD, Rosenfeld RG (eds.), The insulin-like growth factors and their regulatory proteins. Amsterdam: Excerpta Medica International Congress Series 1056, Elsevier Science BV. 1994: 341-349.

Adashi EY, Resnick CE, Svoboda ME, Van Wyk JJ. Somatomedin-C as an amplifier of follicle-stimulating hormone action: enhanced accumulation of adenosine 3',5'-monophosphate. Endocrinology 1986; 118: 149-155.

Adashi EY, Resnick CE, Svoboda ME, Van Wyk JJ. Somatomedin-C enhances induction of luteinizing hormone receptors by follicle-stimulating hormone in cultured rat granulosa cells. Endocrinology 1985; 116: 2369-2375.

Ahima RS, Kelly J, Elmquist JK, Flier JS. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinemia. Endocrinology 1999; 140: 4923-4931.

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. Nature 1996; 382: 250-252.

Ainsworth L, Tsang BK, Downey BR, Marcus GJ. The synthesis and actions of steroids and prostaglandins during follicular maturation in the pig. J Reprod Fertil Suppl 1990; 40: 137-150.

Akinlosotu BA, Diehl JR, Gimenez T. Prostaglandin E2 counteracts the effects of PGF2 alpha in indomethacin treated cycling gilts. Prostaglandins 1988; 35: 81-93.

Almeida FRCL, Kirkwood RN, Aherne FX, Foxcroft GR. Consequences of different patterns of feed intake during the estrous cycle in gilts on subsequent fertility. J Anim Sci 2000a; 78: 1556-1563.

Almeida FRCL, Mao J, Novak S, Cosgrove JR, Foxcroft GR. Effects of patterns of feed restriction and insulin treatment during the luteal phase on reproductive, metabolic and endocrine parameters in cyclic gilts. 2000b; (submitted to J Anim Sci).

Anderson LL. 1978 Growth, protein content and distribution of early pig embryos. Anat Rec 190: 143-154.

Aplin JD. Adhesion molecules in implantation. Rev Reprod 1997; 2: 84-93.

Arias P, Rodriguez M, Szwarcfarb B, Sinay I, Moguilevsky JA. Effect of insulin on LHRH release by perifused hypothalamic fragments. Neuroendocrinology 1992; 56: 415-418.

Armstrong DG, Webb R. Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. Rev Reprod 1997; 2: 139-146.

Armstrong JD, Kraeling R, Britt JH. Effects of naloxone or transient weaning on secretion of LH and prolactin in lactating sows. J Reprod Fertil 1988a; 83: 301-308.

Armstrong JD, Kraeling R, Britt JH. Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of estrus after weaning. J Anim Sci 1988b; 66: 2216-2223.

Ashworth CJ. Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. Anim Reprod Sci 1991a; 26: 311-321.

Ashworth CJ. Embryo development. Pig News and Inf 1991b; 12: 551-554.

Ashworth CJ. Nutritional factors related to embryonic mortality in the domestic species. In: Zavy MT, Geisert RD (eds.), Embryonic Mortality in Domestic Species. Boca Raton, Florida. 1994; 179-194.

Ashworth CJ, Antipatis C, Beattie L. Effect of pre- and post-mating nutritional status on embryo survival and uterine function in the pig. J Reprod Fertil Abstr Ser 1995; 16: Abstract 27.

Ashworth CJ, Pickard AR, Miller SJ, Flint AP, Diehl JR. Comparative studies of conceptus-endometrial interactions in Large White x Landrace and Meishan gilts. Reprod Fertil Dev 1997; 9: 217-225.

Ashworth CJ, Pickard AR. Embryo survival and prolificacy. In: Progress in Pig Science. Nottingham University Press. 1998; 303-325.

Ashworth CJ, Sales DI, Wilmut IW. Evidence of an association between the survival of embryos and the periovulatory plasma progesterone concentration in the ewe. J Reprod Fertil 1989; 87: 23-32.

Bai FL, Yamano M, Shiotani Y, Emson PC, Smith AD, Powell JF, Tahyama M. An arcuato-paraventricular and dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenalin in the rat. Brain Res 1985; 331: 172-175.

Balasubramaniam AA. Neuropeptide Y family of hormones: Receptor subtypes and antagonists. Peptides 1997; 18: 445-457.

Balasubramanian K, Lavoie HA, Garmey JC, Stocco DM, Veldhuis JD. Regulation of porcine granulosa cell steroidogenic acute regulatory protein (StAR) by insulin-like

growth factor I: synergism with follicle-stimulating hormone or protein kinase A agonist. Endocrinology 1997; 138: 433-439.

Banks WA, Kastin AJ, Huang WT, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides 1996; 17: 305-311.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper J, Clifton DK, Steiner KA. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996; 137: 3144-3147.

Bazer FW, Geisert RD, Thatcher WW, Roberts RM. The establishment and maintenance of pregnancy. In: Cole DJA, Foxcroft GR (eds.), Control of Pig Reproduction. London, Butterworths. 1982; 227-252.

Bazer FW, Marengo SR, Geisert RD, Thatcher WW. Exocrine versus endocrine secretion of prostagland F2alpha in the control of pregnancy in swine. Anim Reprod Sci 1984; 7: 115-132.

Bazer FW, Thatcher WW. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2alpha by uterine endometrium. Prostaglandins 1977; 14: 397-400.

Bazer FW, Thatcher WW, Martinat-Botte F, Terqui M. Conceptus development in large white and prolific Chinese Meishan pigs. J Reprod Fertil. 1988; 84: 37-42.

Beck B, Burlet A, Nicolas JP, Burlet C. Unexpected regulation of hypothalamic neuropeptide Y by food deprivation and refeeding in the Zucker rat. Life Sci 1992; 50: 923-930.

Behrman HR, Preston SL. Luteolytic actions of peroxide in rat ovarian cells. Endocrinology 1989; 124: 2895-2900.

Besecke LM, Levine JE. Acuate increase in responsiveness of luteinizing hormone (LH)-releasing hormone nerve terminals to neuropeptide Y stimulation before the preovulatory LH surge. Endocrinology 1994; 135: 63-66.

Besecke LM, Wolfe AM, Poerce ME, Takahashi JS, Levine JE. Neuropeptide Y stimulates luteinizing hormone-releasing hormone release from superfused hypothalamic GT 1-7 cells. Endocrinology 1994; 135: 1621-1627.

Besnard N, Pisselet C, Monniaux D, Monget P. Proteolytic activity degrading insulin-like growth factor-binding protein-2, -3, -4, and -5 in healthy growing and atretic follicles in the pig ovary. Biol Reprod 1997; 56: 1050-1058.

Bicsak TA, Shimonaka M, Malkowski M, Ling N. Insulin-like growth factor-binding protein (IGF-BP) inhibition of granulosa cell function: effect on cyclic adenosine 3',5'-monophosphate, deoxyribonucleic acid synthesis, and comparison with the effect of an IGF-I antibody. Endocrinology 1990; 126: 2184-2189.

Blair RM, Geisert RD, Zavy MT, Short EC, Fulton RW, Yellin T. Endometrial morphology and secretory alteration associated with embryonic mortality in gilts administered estradiol valerate on Days 9 and 10 of gestation. Biol Reprod 1991; 44: 1063-1079.

Booth PJ. Metabolic influences in hypothalamic-pituitary-ovarian function in the pig. J Reprod Fertil Suppl 1990; 40: 89-100.

Booth PJ, Craigon J, Foxcroft GR. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J Anim Sci 1994; 72: 2415-2424

Boukhliq R, Miller DW, Martin GB. Relationship between nutritional stimulation of gonadotropin secretion and the peripheral and cerebrospinal fluid (CSF) concentrations of glucose and insulin in rams. Anim Reprod Sci 1996; 41: 201-214.

Brady LS, Smith MA, Gold PW, Herkenham M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 1992; 52: 441-447.

Brannstrom M, Norman RJ. Involvement of leukocytes and cytokines in the ovulatory process and corpus luteum function. Hum Reprod 1993; 8: 1762-1775.

Briski KP. Central opioid receptors mediate glucoprivic inhibition of pituitary LH secretion. Am J Physiol 1997; 272: E517-522.

Britt JH. Biology and management of the early weaned sow. Biology & Management of the Breeding Herd. Minnesota. 1996; 46-56.

Britt JH, Armstrong J, Cox NM, Esbenshade KL. Control of follicular development during and after lactaton in sows. J Reprod Fertil Suppl 1985; 33: 37-54.

Broermann DM, Xie S, Nephew KP, Pope WF. Limitation of oviductal transfer in swine. Theriogenology 1990; 33: 709-722.

Bronson FH. Puberty in female rats: relative effect of exercise and food restriction. Am J Physiol 1987; 252: R140-R144.

Brown RW, Hungerford JW, Greenwood PE, Bloor RJ, Evans DF, Tsonis CG, Forage RG. Immunization against recombinant bovine inhibin alpha subunit causes increased ovulation rates in gilts. J Reprod Fertil 1990; 90: 199-205.

Bucholtz DC, Manning C, Herbosa CG, Schillo K, Foster DL. The energetic of LH secretion: a temporally-focused view of sexual maturation. 23rd Ann Meet Soie Neurosci, Washington DC 1993; 1: (Abstr. 349).

Bucholtz DC, Vidwans NM, Herbosa CG, Schillo KK, Foster DL. Metabolic interfaces between growth and reproduction. V. Pulsatile luteinizing hormone secretion is dependent on glucose availability. Endocrinology 1996; 137: 601-607.

Calka J, Majewski M, Kalecxyc J, Lakomy M. Immunocytochemical demonstration of neuropeptide Y and luteinizing hormone-releasing hormone-immunoreactive structures in the organum vasculosum laminae terminalis of juvenile gilts. Nuerosci Lett 1993; 158: 21-24.

Cameron JL, Nosbisch C. Suppression of pulsatile luteinizing hormone and testosterone secretion during short term food restriction in the adult male rhesus monkey (Macaca macula). Endocrinology 1991; 128: 1532-1540.

Cameron VA, Nishimura E, Mathews LS, Lewis KA, Sawchenko PE, Vale WW. Hybridization histochemical localization of activin receptor subtypes in rat brain, puitary, ovary, and testis. Endocrinology 1994; 134: 799-808.

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995; 269: 546-549.

Cara JF, Fan J, Azzrello J, Rosenfield RL. Insulin-like growth factor-I enhances luteinizing hormone binding to rat ovarian theca-interstitial cells. J Clin Invest 1990; 86: 560-565.

Carr BR. The ovary. In: Carr BR, Blackwell RE (eds.), Textbook of Reproductive Medicine (2nd ed.) Stamford, Connecticut. 1998; 207-227.

Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, Dieguez C. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology 1997; 66: 375-377.

Carson RS, Zhang Z, Hutchinson LA, Herington AC, Findlay JK. Growth factors in ovarian function. J Reprod Fertil 1989; 85: 735-746.

Cassar G, Chapeau C, King GJ. Effects of increased dietary energy after mating on development uniformity and survival of porcine conceptus. J Anim Sci 1994; 72: 1320-1324.

Catzeflis C, Pierroz DD, Rohner-Jeanrenaud F, Rivier JE, Sizonenko PC, Aubert ML. Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats. Endocrinology 1993; 132: 224-234.

Chang WY, Shidaifat F, Uzumcu M, Lin YC. Effects of transforming growth factor-beta 1 and activin-A on *in vitro* porcine granulosa cell steroidogenesis. Theriogenology 1996; 45: 1463-1472.

Channing C, Kammerman S. Characteristics of gonadotropin receptors of porcine granulosa cells during maturation. Endocrinology 1973; 92: 531-540.

Chedrese PJ, The VL, Labrie F, Juorio AV, Murphy BD. Evidence for the regulation of 3 beta-hydroxysteroid dehydrogenase messenger RNA by human chorionic gonadotropin in luteinized porcine granulosa cells. Endocrinology 1990; 126: 2228-2230.

Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996; 12: 318-320.

Chen MD, O'Byrne KT, Chiappini SE, Hotchkiss J, Knobil E. Hypoglycemic 'stress' and gonadotropin-releasing hormone pulse generator activity in the rhesus monkey: role of the ovary. Neuroendocrinology 1992; 56: 666-673

Chen MD, Ordog T, O'Byrne KT, Goldsmith JR, Connaughton MA, Knobil E. The insulin hypoglycemia-induced inhibition of gonadotropin-releasing hormone pulse generator activity in the rhesus monkey: roles of vasopressin and corticotropin-releasing factor. Endocrinology 1996; 137: 2012-2021.

Chun S, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsueh AJN. Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. Endocrinology 1996; 137: 1447-1456.

Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 1994; 269: 28314-28322.

Clarke IJ, Horton RJE, Doughton BW. Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes. Endocrinology 1990; 127: 1470-1476.

Clowes EJ, Aherne FX, Foxcroft GR. Effect of delayed breeding on the endocrinology and fecundity of sows. J Anim Sci 1994; 72: 283-291.

Conley A, Howard H, Slanger W, Ford J. Steroidogenesis in the preovulatory porcine follicle. Biol Reprod 1994; 51: 655-661.

Conley AJ, Ford SP. Direct luteotropic effect of oestradiol-17beta on pig corpora lutea. J Reprod Fertil 1989; 87: 125-131.

Conley AJ, Kaminski MA, Dubowsky SA, Jablonka-Shariff A, Redmer DA, Reynolds LP. Immuno-histochemical localization of 3 beta-hydroxysteroid dehydrogenase and P450 17 alpha-hydroxylase during follicular and luteal development in pigs, sheep, and cows. Biol Reprod 1995; 52: 1081-1094.

Constantino CX, Keyers PL, Kostyo JL. Insulin-like growth factor-I stimulates steroidogenesis in rabbit luteal cells. Endocrinology 1991; 128: 1702-1708.

Cosgrove JR, Foxcroft GR. Nutrition and reproduction in the pig: ovarian aetiology. Anim Reprod Sci 1996; 42: 131-141.

Couet J, Martel C, Dupont E, Luu-The V, Sirard MA, Zhao HF, Pelletier G, Labrie F. Changes in 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase messenger ribonucleic acid, activity and protein levels during the estrous cycle in the bovine ovary. Endocrinology 1990; 127: 2141-2148.

Cox NM, Barb CR, Kesner JS, Kraeling RR, Matamoros IA, Rampacek GB. Effects of intracerebroventricular (icv) administration of insulin on luteinizing hormone (LH) in gilts. 3rd. Int. Conf. Pig. Reprod. 1989; Abstract 7.

Cox NM, Britt JH. Relationship between endogenous gonadotropin-releasing hormone, gonadotropin, and follicular development after weaning in sows. Biol Reprod 1982a; 27: 70-78.

Cox NM, Britt JH. Pulsatile administration of gonadotropin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus. Biol Reprod 1982b; 27: 1126-1137.

Cox NM, Meurer KA, Carlton CA, Tubbs RC, Mannis DP. Effect of diabetes mellitus during the luteal phase of the oestrous cycle on preovulatory follicular function, ovulation and gonadotrophins in gilts. J Reprod Fertil 1994; 101: 77-86.

Crowley WR, Kalra SP. Regulation of luteinizing hormone secretion by neuropeptide Y in rats: hypothalamic and pituitary actions. Synapse 1988; 2: 276-281.

Dantzer V. Electron microscopy of the initial stages of placentation in the pig. Anat Embryol 1985; 172: 281-293.

Day BN, Polge C. Effects of progesterone on fertilization and egg transport in the pig. J Reprod Fertil 1968; 17: 227-230.

De Rensis F. Neuroendocrine regulation of post-partum anestrus in the sow. Edmonton, CA: University of Alberta; 1993. Ph.D. Thesis.

De Rensis F, Cosgrove JR, Foxcroft GR. Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol Reprod 1993a; 48: 970-976.

De Rensis F, Hunter MG, Foxcroft GR. Suckling-induced inhibition of luteinizing hormone secretion and follicular development in the early post-partum sow. Biol Reprod 1993b; 48: 964-969.

De Rensis F, Foxcroft GR. Correlation between LH response to challenges with GNRH and naloxone during lactation, and LH secretion and follicular development after weaning in the sow. Anim Reprod Sci 1999; 56: 143-152.

De Rensis F, Hunter MG, Grant SA, Lancaster RT, Foxcroft GR. Effect of estrogen administration on endogenous and luteinizing hormone-releasing hormone-induced luteinizing hormone secretion and follicular development in the lactating sow. Biol Reprod 1991; 44: 975-982.

De Vos P, Saladin R, Auwerx J, Staels B. Induction of ob gene expression by corticosteroids is accompanied by body weihgt loss and reduced food intake. J Biol Chem 1995; 270: 15958-15961.

De Winter JP, ten Dijke P, de Vries CJ, van Achterberg TA, Sugino H, de Waele P, Huylebroeck D, Verschueren K, van den Eijnden-van Raaij AJ. Follistatins neutralize activin bioactivity by inhibition of activin binding to its type II receptors. Mol Cell Endocrinol 1996; 116: 105-114.

deMoura MD, Choi D, Adashi EY, Payne DW. Insulin-like growth factor-I-mediated amplification of follicle-stimulating hormone-supported progesterone accumulation by cultured rat granulosa cells: enhancement of steroidogenic enzyme activity and expression. Biol Reprod 1997; 56: 946-953.

den Hartog LA, Vesseur PC, Kemp B. Nutrition-reproduction interaction in sows. In: Cole DJA, Wiseman J, Warley MA (eds.). Priniciples of Pig Science. 1994; 215-224.

Dhindsa DS, Dziuk PJ, Norton HW. Time of transuterine migration and distribution of embryos in the pig. Anat Rec 1967; 159: 325-330.

Dietrich E, Hennies M, Holtz W, Voglmayr J. Immunization of goats against recombinant human inhibin alpha subunit: effect on inhibin binding, mating behaviors, ovarian activity and embryo yield. Anim Reprod Sci 1995; 39: 118-128.

Doldi N, Bassan M, Messa A, Ferrari A. Expression of vascular endothelial growth factor in human luteinizing granulosa cells and its correlation with the response to controlled ovarian hyperstimulation. Gynecol Endocrinol 1997; 11: 263-267.

Dong Q, Bergendahl M, Huhtaniemi I, Handelsman DJ. Effects of undernutrition on pulsatile luteinizing hormone (LH) secretion in castrate and intact male rats using an ultrasensitive immunofluorometric LH assay. Endocrinology 1994; 135: 745-750.

Dong Q, Li B, Rintala H, Blair S, Spaliviero J, Handelsman DJ. LH pulsatility and biopotency during undernutrition in the mature male rat. Am J Physiol 1993; 265: E304-E313.

Driancourt MA, Locatelli A, Prunier A. Effects of gonadotropin deprivation on follicular growth in gilts. Reprod Nutr Dev 1995; 35: 663-673.

Driancourt MA, Quesnel H, Meduri G, Prunier A, Hermier D. Luteinization and proteolysis in ovarian follicles of Meishan and Large White gilts during the preovulatory period. J Reprod Fertil 1998; 114: 287-297.

Dubey AK, Cameron JL, Steiner RA, Plant TM. Inhibition of gonadotropin secretion in castrated male rhesus monkeys (Macular mulatta) induced by dietary restriction: analogy with the prepubertal hiatus of gonadotropin release. Endocrinology 1986; 118: 518-525.

Dufour J, Cahill LP, Mauleon P. Short- and long-term effects of hypophysectomy and unilateral ovariectomy on ovarian follicular populations in sheep. J Reprod Fertil 1979; 57: 301-309.

Duleba AJ, Spaczynski RZ, Olive DL, Behrman HR. Effects of insulin and insulin-like growth factors on proliferation of rat ovarian theca-interstitial cells. Biol Reprod 1997; 56: 891-897.

Duleba AJ, Spaczynski RZ, Olive DL. Insulin and insulin-like growth factor I stimulate the proliferation of human ovarian theca-interstitial cells. Fertil Steril 1998; 69: 335-340.

Dumon Y, Fournier A, Pierre S St., Quirion R. Comparative characterization and autoradiographic distribution of neuropeptide Y receptor sites in the rat brain. J Neurosci 1993; 13: 73-86.

Dusza L, Tilton JE. Role of prolactin in the regulation of ovarian function in pigs. J Reprod Fertil Suppl 1990; 40: 33-45.

Dziuk PJ. Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival. J Reprod Fertil Suppl 1985; 33: 57-63.

Dziuk PJ. Embryonic loss in the pig: an enigma. Managing Pig Production. Proceeding Inaugural Conference, Australia, Pig Science Association. 1987; 28-39.

Dziuk PJ, Polge C, Rowson LE. Intra-uterine migration and mixing of embryos in swine following egg transfer. J Anim Sci 1964; 23: 37-42.

Edwards JL, Hughey TC, Moore AB, Cox NM. Depletion of insulin in streptozocin-induced-diabetic pigs alters estradiol, insulin-like growth factor (IGF)-I and IGF binding proteins in cultured ovarian follicles. Biol Reprod 1996; 55: 775-781.

Edwards S. The endocrinology of the post-partum sow. In: Cole DJA, Foxcroft GR (eds.), Control of Pig Reproduction. London, Butterworths. 1982; 439-458.

Edwards S, Foxcroft GR. Endocrine changes in sows weaned at two stages of lactation. J Reprod Fertil 1983a; 67: 161-172.

Edwards S, Foxcroft GR. Response of sows to oestradiol benzoate treatment after weaning at two stages of lactation. J Reprod Fertil 1983b; 67: 173-180.

Erickson GF, Garzo VG, Magoffin DA. Progesterone production by human granulosa cells cultured in serum free medium: effects of gonadotrophins and insulin-like growth factor I (IGF-1). Hum Reprod 1991; 6: 1074-1081.

Erickson JC, Hollopeter G, Palmiter RD. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 1996; 274: 1704-1707.

Evans G, Dobias M, King GJ, Armstrong DT. Estrogen, androgen, and progesterone biosynthesis by theca and granulosa of preovulatory follicles in the pig. Biol Reprod. 1981; 25: 673-682.

Faillace L, Hunter M. Follicle development and oocyte maturation during the immediate preovulatory period in Meishan and White hybrid gilts. J Reprod Fertil 1994; 101: 571-576.

Feng SM, Almond GW. Identification and distribution of prostaglandin E receptors on porcine luteal cells. Biol Reprod 1996; 54: 1366-1374.

Feng SM, Almond GW. Effects of LH, prostaglandin E2, 8-bromo-cyclic AMP and forskolin on progesterone secretion by pig luteal cells. J Reprod Fertil 1998; 113: 83-89.

Feng SM, Almond GW. PGE receptor characteristics on porcine luteal cells during the estrous cycle and early pregnancy. Prostaglandins Other Lipid Mediators 1999; 57: 13-22.

Ferrara N, Chen H, Davis-Smyth T, Gerber HP, Nguyen TN, Peers D, Chisholm V, Hillan KJ, Schwall RH. Vascular endothelial growth factor is essential for corpus luteum angiogenesis. Nat Med 1998; 4: 336-340.

Fielder PJ, Pham H, Adashi EY, Rosenfeld RG. Insulin-like growth factor (IGFs) block FSH-induced proteolysis of IGF-binding protein-5 (BP-5) in cultured rat granulosa cells. Endocrinology 1993; 133: 415-418.

Flint AP, Burton RD, Gadsby JE, Heap RB, Sheldrick EL. Embryonic steroid synthesis and luteal oxytocin production: controlling mechanisms for the maternal recognition of pregnancy. J Steroid Biochem 1983; 19: 973-978.

Ford JJ, Howard HJ. Activin inhibition of estradiol and progesterone production in porcine granulosa cells. J Anim Sci 1997; 75: 765-766.

Ford SP, Magness RR, Farley DB, Van Orden DE. Local and systemic effects of intrauterine estradiol-17beta on luteal function of nonpregnant sows. J Anim Sci 1982; 55: 657-660.

Formigoni A, Parmeggiani A, Spinaci M, Govoni N, Galeati. Influence of the energy source on pulsatile LH secretion in prepubertal gilts. Proc. 14th IPVS Cong. Bologna, Italy. 1996.

Foxcroft GR. Interactions between body condition, metabolic state and reproduction in swine. Proc. 11th West Nutrit. Conf. Faculty of Extension, University of Alberta. 1990; 1-8.

Foxcroft GR. Nutritional and lactational regulation of fertility in sows. J Reprod Fertil Suppl 1992; 45: 113-125.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Foxcroft GR, Aherne FX, Clowes EJ, Miller HM, Zak LJ. Sow fertility: the role of suckling inhibition and metabolic status. In: Ivan M (ed). Animal science research and development: Moving towards a new century. Ottawa, Canada. 1995; 377-391.

Foxcroft GR, Cosgrove JR, Ding J, Hofacker S, Wiesak T. Reproductive function: Current concepts. In: Cole DA, Wiseman J, Valey MA (eds.), Principles of Pig Science. Nottingham: Nottingham University Press. 1994; 225-252.

Foxcroft GR, Hunter MG. Basic physiology of follicular maturation in the pig. J Reprod Fertil Suppl 1985; 33: 1-19.

Foxcroft GR, Shaw HJ, Hunter MG, Booth PJ, Lancaster RT. Relationship between luteinizing hormone, follicle stimulating hormone and prolactin secretion and ovarian follicular development in the weaned sow. Biol Reprod 1987; 36: 175-191.

Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1995; 1: 1311-1314.

Gadsby JE, Lovdal JA, Samaras S, Barber JS. Hammond JM. Expression of the messenger ribonucleic acids for insulin-like growth factor-I and insulin-like growth factor binding proteins in porcine corpora lutea. Biol Reprod 1996; 54: 339-346.

Gadsby JE, Smith CA, Almond GW. Acute stimulatory effects of prostaglandin F2 alpha on serum progesterone concentrations in pregnant and pseudopregnant pigs. Prostaglandins 1991; 41: 419-432.

Gebarowska D, Ziecik AJ, Gregoraszczuk EL. Luteinizing hormone receptors on granulosa cells from preovulatory follicles and luteal cells throughout the oestrous cycle of pigs. Anim Reprod Sci 1997; 49: 191-205.

Geisert RD, Renegar RH, Thatcher WW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: I. Interrelationshipd between preimplantation development of the pig blastocyst and uterine endometrial secretion. Biol Reprod 1982a; 27: 925-939.

Geisert RD, Brookbank JW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol Reprod 1982b; 27: 941-955.

Geisert RD, Morgan GL, Zavy MT, Blair RM, Gries LK, Cox A, Yellin T. Effect of asynchronous transfer and oestrogen administration on survival and development of porcine embryos. J Reprod Fertil 1991; 93: 475-481.

Geisert RD, Zavy MT, Moffatt RJ, Blair RM, Yellin T. Embryonic steroids and the establishment of pregnancy in pigs. J Reprod Fertil Suppl 1990; 40: 293-305.

Geisert RD, Zavy MT, Wettemann RP, Biggers. Length of pseudopregnancy and pattern of uterine protein released as influenced by time and duration of estrogen administration in the pig. J Reprod Fertil 1987; 79: 163-172.

Goldstein MH, Bazer FW, Barrow DH. Characterization of changes in volume, osmolarity and electrolyte composition of porcine fetal fluids during gestation. Biol Reprod 1980; 22: 1168-1180.

Gore-Landton RE, Armstrong DT. Follicular steroidogenesis and its control. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction. New York, Raven Press. 1988; 331-385.

Gougeon A. Regulation of ovarian follicular development in primates: facts and hypothesis. Endocr Rev 1996; 17: 121-155.

Grant S. Control of follicular development in the cyclic gilt and weaned sow. Nottingham, UK: University of Nottingham. 1989; Ph.D. Thesis.

Grant SA, Hunter MG, Foxcroft GR. Morphological and biochemical characteristics during ovarian follicular development in the pig. J Reprod Fertil 1989; 86: 171-183.

Gregoraszczuk EL, Michas N. Progesterone and estradiol secretion by porcine luteal cells is influenced by individual and combined treatment with prostaglandins E2 and F2alpha throughout the estrus cycle. Prostaglandins Other Lipid Mediators 1999; 57: 231-241.

Gries LK, Geisert RD, Zavy MT, Garret JE, Morgan GL. Uterine secretion alteration coincident with embryonic mortality in the gilt after exogenous estrogen administration. J Anim Sci 1989; 67: 276-284.

Grimes RW, Barber JA, Shimasaki S, Ling N, Hammond JM. Porcine ovarian granulosa cells secrete insulin-like growth factor-binding proteins-4 and -5 and express their messenger ribonucleic acids: regulation by follicle-stimulating hormone and insulin-like growth factor-1. Biol Reprod 1994; 50: 695-701.

Grimes RW, Hammond JM. Insulin and insulin-like growth factors (IGFs) stimulate production of IGF-binding proteins by ovarian granulosa cells. Endocrinology 1992; 131: 553-558.

Grimes RW, Hammond JM. Proteolytic degradation of insulin-like growth factor (IGF)-binding protein-3 by porcine ovarian granulosa cells in culture: Regulation by IGF-I. Endocrinology 1994; 134: 337-343.

Grimes RW, Samaras SE, Barber JA, Shimasaki S, Ling N, Hammond JM. Gonadotropin and cAMP modulation of IGE binding protein production in ovarian granulosa cells. Am J Physiol 1992; 262: E497-E503.

Gross TS, Lacroix MC, Bazer FW, Thatcher WW, Harney JP. Prostaglandin secretion by perifused porcine endometrium: further evidence for endocrine versus exocrine secretion of prostaglandins Prostaglandins 1988; 35: 327-341.

Guthrie HD, Cooper BS, Welch GR, Zakaria AD, Johnson LA. Atresia in follicles grown after ovulation in the pig: measurement of increased apoptosis in granulosa cells and reduced follicular fluid estradiol-17beta. Biol Reprod 1995; 52: 920-927.

Guthrie HD, Garrett WM, Cooper BS. Follicle-stimulating hormone and insulin-like growth factor-I attenuate apoptosis in cultured porcine granulosa cells. Biol Reprod 1998; 58: 390-396.

Guthrie HD, Pursel VG, Frobish LT. Attempts to induce conception in lactating sows. J Anim Sci 1978; 47: 1145-1151.

Hammond JM, Samaras SE, Grimes R, Leighton J, Barber J, Canning SF, Guithrie HD. The role of insulin-like growth factors and epidermal growth factor-related peptides in intraovarian regulation in the pig ovary. J Reprod Fertil Suppl 1993; 48: 117-125.

Harlow CR, Shaw HJ, Hillier SG, Hodges JK. Factors influencing follicle-stimulating hormone-responsive steroidogenesis in marmoset granulosa cells: effects of androgens and the stage of follicular maturity. Endocrinology 1986; 122: 2780-2787.

Hasegawa Y, Miyamoto K, Iwamura S, Igarashi M. Changes in serum concentrations of inhibin in cyclic pigs. J Endocrinol 1988; 118: 211-219.

Heap RB, Flint APF, Gadsby JE, Rice C. Hormones, the early embryo and the uterine environment. J Reprod Fertil 1979; 55: 267-275.

Hedin L. Rodgers RJ. Simpson ER. Richards JS. Changes in content of cytochrome P450(17)alpha, cytochrome P450scc, and 3-hydroxy-3-methylglutaryl CoA reductase in developing rat ovarian follicles and corpora lutea: correlation with theca cell steroidogenesis. Biol Reprod 1987; 37: 211-223.

Heilig M, Widerlov E. Neurobiology and clinical aspects of neuropeptide Y. Crit Rev Neurobiol 1995; 9: 115-136.

Hickey GJ, SA Chen, MJ Besman, JE Shively, PF Hall, DG Kurten, JS Richards. Hormonal regulation, tissue distribution, and content of aromatase cytochrome P450 messenger ribonucleic acid and enzyme in rat ovarian follicles and corpora lutea: relationship to estradiol biosynthesis. Endocrinology 1988; 122: 1426-1436.

Hileman SM, Schillo KK, Hall JB. Effects of acute, intracerebroventricular administration of insulin on serum concentrations of luteinizing hormone, insulin, and glucose in ovariectomized lambs during restricted and ad libitum feed intake. Biol Reprod 1993; 48: 117-124.

Hillier SG. Regulatory functions for inhibin and activin in human ovaries. J Endocrinol 1991; 131: 171-175.

Hillier SG, Reichert LE, van Hall EV. Control of preovulatory follicular estrogen biosynthesis in the human ovary. J Clin Endocrinol Metab 1981; 52: 847-856.

Hillier SG, Whitelaw PF, Smyth CD. Follicular estrogen synthesis: the 'two-cell, two-gonadotropin' model revisited. Mol Cell Endocrinol 1994; 100: 51-54.

Howard H, Ford J. Relationships among concentrations of steroids, inhibin, insulin-like growth factor-1 (IGF-1), and IGF binding proteins during follicular development in weaned sows. Biol Reprod 1992; 47: 193-201.

Hsueh AJ, Adashi EY, Jones PB, Welsh TH Jr. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr Rev 1984; 5: 76-127.

Hsueh AJW, Dahl KD, Vaughan J, Tucker E, Rivier J, Bardin CW, Vale W. Heterodimers and homodimers of inhibin subunits have different paracrine action in the modulation of luteinizing hormone-stimulated androgen biosynthesis. Proc Natl Acad Sci USA 1987; 84: 5082-5086.

Huang CJ, Li Y, Stromer MH, Anderson LL. Synergistic effects of insulin-like growth factor I and gonadotrophins on relaxin and progesterone secretion by aging corpora lutea of pigs. J Reprod Fertil 1992; 96: 415-425.

Hunter MG. Responsiveness *in vitro* of porcine luteal tissue recovered at two stages of the luteal phase. J Reprod Fertil 1981; 63: 471-476.

Hunter MG, Grant S, Foxcroft GR. Histological evidence for heterogenity in the development of preovulatory pig follicles. J Reprod Fertil 1989; 86: 165-170.

Hunter MG, Picton HM, Biggs C, Mann GE, McNeilly AS, Foxcroft GR. Periovulatory endocrinology in high ovulating Meishan sows. J Endocrinol 1996; 150: 141-147.

Hunter MG, Picton HM. Effect of hCG administration at the onset of estrus on early embryo survival and development in Meishan gilts. Anim Reprod Sci 1995; 38: 231-238.

Hunter MG, Wiesak T. Evidence for and implications of follicular heterogeneity in pigs. J Reprod Fertil Suppl 1990; 40: 163-177.

Hunter RHF. Physiological factors influencing ovulation, fertilization, early embryonic development and establishment of pregnancy in pigs. Br Vet J 1977; 133: 461-470.

I'Anson H, Foster DL, Booth PJ, Foxcroft GR. Nutritional status and reproduction. Oxford Rev Reprod Biol 1991; 13:239-311.

l'Anson H, Manning JM, Herbosa CG, Pelt J, Friedman CR, Wood RI, Bucholtz DC, Foster DL. Central inhibition of gonadotropin-releasing hormone secretion in the growth-restricted hypogonadotropic female sheep. Endocrinology 2000; 141: 520-527.

Jain MR, Pu S, Kalra PS, Kalra SP. Evidence that stimulation of two modalities of pituitary luteinizing hormone release in ovarian steroid-primed ovariectomized rats may involve neuropeptide Y Y1 and Y4 receptors. Endocrinology 1999; 140: 5171-5177.

Jarrell VL, Beckmann LS, Cantley TC, Rieke AR, Day BN. The effect of exposure to an asynchronous uterus on development of day 4 swine embryos. J Anim Sci 1990; 68(suppl. 1): 428

Jarry H, Einspanier A, Kanngiesser L, Dietrich M, Pitzel L, Holtz W, Wuttke W. Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an *in vivo* microdialysis system. Endocrinology 1990; 126: 2350-2358.

Jin M, Lu J, Wan TC, Wu C. Inhibin subunit gene expression and distribution in the ovaries of immature, young, adult, middle aged and old female rats. Endocrinology 1993; 132: 319-326.

Jindal, R. Physiological mechanisms mediating nutritional effects on embryonic survival in the pig. Edmonton, CA: University of Alberta, 1996. Ph.D. Thesis.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. J Anim Sci 1996; 74: 620-624.

Jindal R, Cosgrove JR, Foxcroft GR. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. J Anim Sci 1997; 75: 1063-1070.

Johnston LJ, Pettigrew JE, Rust JW. Response of maternal-line sows to dietary protein concentration during lactation. J Anim Sci 1993; 71: 2151-2156.

Kalra PS, Bonavera JJ, Kalra SP. Central administration of antisense oligodeoxynucleotides to neuropeptide Y (NPY) mRNA reveals the critical role of newly synthesized NPY in regulation of LHRH release. Regul Pept 1995; 59: 215-220.

Kalra SP. Neural circuitry involved in the control of LHRH secretion: a model for preovulatory LH release. Front Neuroendocrinol 1986; 9: 31-75.

Kalra SP. Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. Endocr Rev 1993; 14: 507-538.

Kalra SP, Crowley WR. Norepinephrine-like effects of neuropeptide Y on LH release in rat. Life Sci 1984; 35: 1173-1176.

Kalra SP, Crowley WR. Neuropeptide Y: a novel neuroendocrine peptide in the control of pituitary hormone secretion, and its relation to luteinizing hormone. Front Neuroendocrinol 1992; 13: 1-36.

Kalra SP, Fuentes M, Fournier A, Parker SL, Crowley WR. Involvement of the Y-1 receptor subtype in the regulation of luteinizing hormone secretoin by neuropeptide Y in rats. Endocrinology 1992; 130: 3323-3330.

Kalra SP, Kalra PS. Neural regulation of luteinizing hormone secretion in the rat. Endocr Rev 1983; 4: 311-351.

Kalra SP, Kalra PS. Nutritional infertility: the role of the interconnected hypothalamic neuropeptide Y-galanin-opioid network. Front Neuroendocrinol 1996; 17: 371-401.

Kalra SP, Xu B, Dube MG, Moldawer LL, Martin D, Kalra PS. Leptin and ciliary neurotropic factor (CNTF) inhibit fasting-induced suppression of luteinizing hormone release in rats: role of neuropeptide Y. Neurosci Lett 1998; 240: 45-49.

Kaynard AH, Pau KY, Hess DL, Spies HG. Third-ventricular infusion of neuropeptide Y suppresses luteinizing hormone secretion in ovariectomized rhesus macaques. Endocrinology 1990; 127: 2437-2444.

Kemp B, Soede NM. Relationship of weaning-to-estrus interval to timing of ovulation and fertilization in sows. J Anim Sci 1996; 74: 944-949.

Keys JL, King GJ. Effect of intraluminal application and systemic administration of estradiol on porcine endometrial morphology. Biol. Repod. Suppl. 1988; 38: 132.

Khorram O, Pau EKY, Spies HG. Bimodal effects of neuropeptide Y on hypothalamic release of gonadotropin releasing hormone in conscious rabbits. Neuroendocrinology 1987; 45: 290-297.

King BF, Britt JH, Esbenshade KL, Flowers WL, Ireland JJ. Evidence for a local role of inhibin or inhibin alpha subunits in compensatory ovarian hypertrophy. J Reprod Fertil 1995; 104: 291-295.

King RH, Williams IH. The effect of nutrition on the reproductive performance of first-litter sows. 1. Feeding level during lactation and between weaning and remating. Anim Prod 1984a; 38: 241-247.

King RH, Williams IH. The effect of nutrition on the reproductive performance of first-litter sows. 2. Protein and energy intakes during lactation. Anim Prod 1984b; 38: 249-256.

Knight PG. Roles of inhibins, activins, and follistatin in the female reproductive system. Front Neuroendocrinol 1996; 17: 476-509.

Koketsu Y, Dial GD, Pettigrew JE, Marsh WE, King VL. Influence of imposed feed intake patterns during lactation on reproductive performance and on circulating levels of glucose, insulin and luteinizing hormone in primiparous sows. J Anim Sci 1996; 74: 1036-1046.

Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. Acute and chronic effects of insulin on leptin production in human: studies *in vivo* and *in vitro*. Diabetes 1996; 45: 699-701.

Kraeling RR, Barb CR. Hypothalamic control of gonadotrophin and prolactin secretion in pigs. J Reprod Fertil Suppl 1990; 40: 3-17.

Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997; 15: 201-204.

LaBarbera AK. Follicle-stimulating hormone (FSH) receptors and FSH-receptor adenosine 3', 5'-cyclic monophosphate production in porcine granulosa cells decline with follicular growth. Endocr Res 1994; 20: 65-77.

Landefeld TD, Ebling FJ, Suttie LM, Vannerson LA, Padmanabhan V, Beitins IZ, Foster DL. Metabolic interfaces between growth and reproduction. Endocrinology 1989; 125: 351-356.

LaPolt P, Soto D, Su JG, Campen C, Vaughan J, Vale W, Hsueh A. Activin stimulation of inhibin secretion and messenger RNA levels in cultured granulosa cells. Mol Endocrinol 1989; 3: 1666-1673.

Lauderdale JW, Kirkpatrick RL, First NL, Hauser ER, Casida LE. Ovarian and pituitary gland changes in periparturient sows. J Anim Sci 1965; 24: 1100-1103.

Lee A, Christenson LK, Patton PE, Burry KA, Stouffer RL. Vascular endothelial growth factor production by human luteinized granulosa cells *in vitro*. Hum Reprod 1997; 12: 2756-2761.

Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. Nature 1996; 379: 632-635.

Lei ZM, Chegini N, Rao ChV. Quantitative cell composition of human and bovine corpora lutea from various reproductive states. Biol Reprod 1991; 44: 1148-1156.

Leighton JK, Grimes RW, Canning SF. Hammond JM. IGF-binding proteins are differentially regulated in an ovarian granulosa cell line. Mol Cell Endocrinol 1994; 106: 75-80.

Lemon M, Loir M. Steroid release *in vitro* by two luteal cell types in the corpus luteum of the pregnant sow. J Endocrinol 1977; 72: 351-359.

Lemon M, Mauleon P. Interaction between two luteal cell types from the corpus luteum of the sow in progesterone synthesis *in vitro*. J Reprod Fertil 1982; 64: 315-323.

Less M, Wolfenson D, Shaham A, Roth Z, Meidan R. Effects of LH surge pattern on progesterone secretion during development of the corpus luteum and *in vitro* luteinization of granulosa cells in cows. J Reprod Fertil Abstr Ser 1998; 21: 33 (Abstract 76).

Leupen SM, Besecke LM, Levine JE. Neuropeptide Y Y1-receptor stimulation is required for physiological amplification of preovulatory luteinizing hormone surges. Endocrinology 1997; 138: 2735-2739.

Li R, Phillip DM, Mather JP. Activin promotes ovarian follicle development *in vitro*. Endocrinology 1995; 136: 849-856.

Lindsell CE, Misra V, Murphy BD. Regulation of follistatin messenger ribonucleic acid in porcine granulosa cells by epidermal growth factor and the protein kinase-C pathway. Endocrinology 1993; 132: 1630-1636.

Liu J, Aronow BJ, Witte DP, Pope WF, La Barbera AR. Cyclic and maturation-dependent regulation of follicle-stimulating hormone receptor and luteinizing hormone receptor messenger ribonucleic acid expression in the porcine ovary. Biol Reprod 1998; 58: 648-658.

Livingstone C, Lyall H, Gould G. Hypothalamic GLUT 4 expression: a glucose and insulin sensing mechanism? Mol Cell Endocrinol 1995; 107: 67-70.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995; 1: 1155-1161.

Magoffin DA, Kurtz KM, Erickson GF. Insulin-like growth factor-I selectively stimulates cholesterol side-chain cleavage expression in ovarian theca-interstitial cells. Mol Endocrinol 1990; 4: 489-496.

Marks JL, Eastman CJ. Ontogeny of insulin binding in different region of the rat brain. Dev Neurosci 1990; 12: 349-358.

Mason HD, Margara R, Winston RML, Seppela M, Koistinen R, Franks S. Insulin-like growth factor-1 (IGF-1) inhibits production of IGF-binding protein-1 while stimulating estradiol secretion in granulosa cells from normal and polycystic human ovaries. J Clin Endocrinol Metab 1993; 76: 1275-1279.

Mason HD, Willis D, Holly JMP, Cwyfan-Hughes SC, Seppala M, Fanks S. Inhibitory effects of insulin-like growth factor-binding proteins on steroidogenesis by human granulosa cells in cultre. Mol Cell Endocrinol 1992; 89: R1-R4.

Matamoros IA, Cox NM, Moore AB. Exogenous insulin and additional energy affect follicular distribution, follicular steroid concentrations, and granulosa cell human chorionic gonadotropin binding in swine. Biol Reprod 1990; 43: 1-7.

Matamoros IA, Cox NM, Moore AB. Effects of exogenous insulin and body condition on metabolic hormones and gonadotropin-induced follicular development in prepuberal gilts. J Anim Sci 1991; 69: 2081-2091.

Mathews L. Activin receptors and cellular signalling by the receptor serine kinase family. Endocr Rev 1994; 15: 310-325.

McArdle CA, Kohl C, Rieger K, Groner I, Wehrenberg U. Effects of gonadotropins, insulin and insulin-like growth factor I on ovarian oxytocin and progesterone production. Mol Cell Endocrinol 1991; 78: 211-220.

McDonald JK. NPY and related substances. Crit Rev Neurobiol 1988; 4: 97-135.

McDonald JK, Lumpkin MD, DePaolo LV. Neuropeptide-Y suppresses pulsatile secretion of luteinizing hormone in ovariectomized rats: possible site of action. Endocrinology 1989; 125: 186-. 191.

McGee E, Sawetawan C, Bird I, Rainey WE, Carr BR. The effects of insulin on 3 beta-hydroxysteroid dehydrogenase expression in human luteinized granulosa cells. J Soc Gynecol Invest 1995; 2: 535-541.

McShane TM, May JL, Keisler DH. Central action of neuropeptide Y may provide a neuromodulatory link between nutrition and reproduction. Biol Reprod 1992; 46: 1151-1157.

McShane TM, Petersen SL, McCrone S, Keisler DH. Influence of food restriction on neuropeptide Y, proopiomelanocortin, and luteiniziing hormone-releasing hormone gene expression in sheep hypothalami. Biol Reprod 1993; 49: 831-839.

Medina CL, Nagatani S, Darling TA, Bucholtz DC, Tsukamura H, Maeda K, Foster DL. Glucose availability modulates the timing of the luteinizing hormone surge in the ewe. J Neuroendocrinol 1998; 10: 785-792.

Meduri G, Vu Hai MT, Jolivet A, Takemori S, Kominami S, Driancourt MA, Milgrom E. Comparison of cellular distribution of LH receptors and steroidogenic enzymes in the porcine ovary. J Endocrinol 1996; 148: 435-446.

Meduri G, Vuhai-Luuthi MT, Jolivet A, Milgrom E. New functional zonation in the ovary as shown by immunohistochemistry of luteinizing hormone receptor. Endocrinology 1992; 131: 366-373.

Mendiola J, Janzen M, Cruz M, Louis CF. Cloning and tissue distribution of leptin mRNA in the pig. Anim Biotech 1997; 8: 227-236.

Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. J Neuroendocrinol 1996; 8: 733-735.

Mercer JG, Lawrence CB, Beck B, Burlet A, Atkinson T, Barrett P. Hypothalamic NPY and prepro-NPY mRNA in Djungarian hamsters: effects of food deprivation and photoperiod. Am J Physiol 1995; 169: R1099-1106.

Meurer KA, Cox NM, Matamoros IA, Tubbs RC. Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J Reprod Fertil 1991a; 91: 187-196.

Meurer KA, Cox NM, Tubbs RC. Absence of insulin affects recruitment and maintenance of preovulatory follicles in cyclic diabetic gilts. J Anim Sci 1991b; 68(Suppl. 1): 466.

Michel U, McMaster J, Findlay J. Regulation of steady-state follistatin message RNA levels in rat granulosa cells *in vitro*. J. Mol. Endocrinol. 1992; 9: 147-156.

Miller DW, Blache D, Boukhliq R, Curlewis JD, Martin GB. Central metabolic messengers and the effects of nutrition on gonadotropin secretion in sheep. J Reprod Fertil 1998; 112: 347-356.

Miller DW, Blache D, Martin GB. The role of intracerebral insulin in the effect of nutrition on gonadotropin secretion in mature male sheep. J Endocrinol 1995; 47: 321-329.

Miller WL. Molecular biology of steroid synthesis. Endocr Rev 1988; 9: 295-318.

Miner DL, Della-Fera MA, Patterson JA, Baile CA. Lateral cerebroventricular injection of neuropeptide Y stimulates feeding in sheep. Am J Physiol 1989; 257: R383-R387.

Miro F, Hillier SG. Modulation of granulosa cell deoxyribonucleic acid synthesis and differentiation by activin. Endocrinology 1996; 137: 464-468.

Miro F, Smyth CD, Hillier SG. Development-related effects of recombinant activin on steroid synthesis in rat granulosa cells. Endocrinology 1991; 129: 3388-3394.

Mondschein JS, Etherton TD, Hammond JM. Characterization of insulin-like growth factor-binding proteins of porcine ovarian follicular fluid. Biol Reprod 1991; 44: 315-320.

Morbeck D, Esbenshade K, Flowers W, Britt J. Kinetics of follicle growth in oocytes. Mol Reprod Dev 1992; 27: 366-375.

Morgan GL, Geisert RD, Zavy MT, Fazleabas AT. Development and survival of pig blastocysts after oestrogen administration on Day 9 or Days 9 and 10 of pregnancy. J Reprod Fertil 1987; 80: 133-144.

Mulheron GW, Wise TH, Stone RT, Ford JJ. Follicle-stimulating hormone regulation of cytochome P-450 side-chain cleavage message ribonucleic acid accumulation by porcine granulosa cells isolated from small and medium follicles. Biol Reprod 1990; 43: 828-834.

Mullan BP, Close WH, Foxcroft GR. Metabolic state of the lactating sow influences plasma LH and FSH before and after weaning. In: Batterham ES (ed.).Manipulating Pig Reproduction III. Australia Pig Science Association, Attwood, VIC, Australia. 1991; 32.

Murahashi K, Bucholtz DC, Nagatani S. Suppression of luteinizing hormone pulses by restriction of glucose availability is mediated by sensors in the brain stem. Endocrinology 1996; 137: 1171-1176.

Murray MK, DeSouza MM, Messinger SM. Oviduct during early pregnancy: hormonal regulation and interactions with the fertilized ovum. Microsc Res Tech 1995; 31: 497-506

Nagatani S, Bucholtz DC, Murahashi K, Estacio MAC, Tsukamura H, Foster DL, Maeda K. Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. Endocrinology 1996; 137: 1166-1170.

Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda KI, Foster DL. Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology 1998; 67: 370-376.

Nakamura T, Asashima M, Eto Y. Isolation and characterization of native activin B. J Biol Chem 1992; 267: 16385-16389.

Nakano R, Akahori T, Katayama K, Tojo S. Binding of LH and FSH to porcine granulosa cells during follicular maturation. J Reprod Fertil 1977; 51: 23-27.

Nakatani A, Shimasaki S, DePaolo LV, Erickson GF, Ling N. Cyclic changes in follistatin messenger ribonucleic acid and its protein in the rat ovary during the estrous cycle. Endocrinology 1991; 129: 603-611.

Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 1998; 83: 2001-2005.

Niswender GD, Juengel JL, McGuire WJ, Belfiore CJ, Wiltbank MC. Luteal function: the estrous cycle and early pregnancy. Biol Reprod 1994; 50: 239-247.

Ober JA, Malven PV. Effect of growth retardation on pituitary luteinizing hormone and hypothalamic neuropeptide Y in ovariectomized sheep. Neuroendocrinology 1992; 56: 331-339.

O'Callaghan D, H Yaakub, LJ Spicer, MP Boland. Effect of nutrition and superovulation on steroids, insulin-like growth factors and insulin-like growth factor binding protein in pre-ovulatory follicles of ewes. J Reprod Fertil Abstr Ser 1998; 21: 24.

Ohleth MK, Bagnell CA. Relaxin secretion and gene expression in porcine granulosa and theca cells are stimulated during *in vitro* luteinization. Biol Reprod 1999; 60: 499-507.

Oliver JE, Aitman TJ, Powell JF, Wilson CA, Clayton RN. Insulin-like growth factor I gene expression in the rat ovary is confined to the granulosa cells of developing follicles. Endocrinology 1989; 124: 2671-2679.

Oxenreider SL, Day BN. Transport and cleavage of ova in swine. J Anim Sci 1965; 24: 413-417.

Palmer WM, Teague HS, Venzke WG. Histological changes in the reproductive tract of the sow during lactation and early postweaning. J Anim Sci 1965; 24: 1117-1422.

Parfitt D, Church K, Cameron JL. Restoration of pulsatile luteinizing hormone secretion after fasting in rhesus monkeys: dependence on size of refed meal. Endocrinology 1991; 129: 749-756.

Parmer TG, Roberts CT Jr, LeRoith D, Adashi EY, Khan I, Solan N, Nelson S, Zilberstein M, Gibori G. Expression, action and steroidal regulation of insulin-like growth factor-I (IGF-I) and IGF-I receptor in the rat corpus luteum: their differential role in the two cell populations forming the corpus luteum. Endocrinology 1991; 129: 2924-2932.

Parr RA, Davis IF, Fairclough RJ, Miles MA. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. J Reprod Fertil 1987; 80: 317-320.

Parr RA, Hughes PE, Davis IF, Miles MA, Ferlazza JC. Nutrition, progesterone and the early pig embryo. Proc 4th Int Conf Pig Reprod, Columbia. 1993; 93 (Abstract).

Pate JL. Regulation of prostaglandin synthesis by progesterone in the bovine corpus luteum. Prostaglandins 1988; 36: 303-315.

Pate JL. Involvement of immune cells in regulation of ovarian function. J Reprod Fertil Suppl 1995; 49: 365-377.

Pate JL. Intercellular communication in the bovine corpus luteum. Theriogenology 1996; 45: 1381-1397.

Perry JS, Rowlands IW. Early pregnancy in the pig. J Reprod Fertil 1962; 4: 175-188.

Pescador N, Houde A, Stocco DM, Murphy BD. Follicle-stimulating hormone and intracellular second messengers regulate steroidogenic acute regulatory protein messenger ribonucleic acid in luteinized porcine granulosa cells. Biol Reprod 1997; 57: 660-668.

Pescador N, Stocco DM, Murphy BD. Growth factor modulation of steroidogenic acute regulatory protein and luteinization in the pig ovary. Biol Reprod 1999; 60: 1453-1461.

Pharazyn A, den Hartog LA, Foxcroft GR, Aherne FX. Dietary energy and protein intake, plasma progesterone and embryo survival in early pregnancy in the gilt. Can. J Anim Sci 1991; 71: 949-952.

Pickard AR, Ashworth CJ. Oestradiol-17beta secretion by Chinese Meishan and Large White X Landrace conceptuses. J Reprod Fertil Abstr Ser 1995; 15: 49.

Pierroz DD, Cazeflis C, Aebi A, Rivier JE, Aubert ML. Chronic administration of nuuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor 1 secretion in intact adult male rats. Endocrinology 1996; 137: 3-12.

Pierroz DD, Gruaz NM, d'Alieves V, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinology 1995; 61: 293-300.

Pitzel L, Jarry H, Wuttke W. Demonstration of oxytocin receptors in porcine corpora lutea: effects of the cycle stage and the distribution on small and large luteal cells. Biol Reprod 1993a; 48: 640-646.

Pitzel L, Jarry H, Wuttke W. Different steroidogenic response of young and aged porcine small and large luteal cells to prostaglandin F2 alpha, oxytocin and estradiol. Exp Clin Endocrinol 1993b; 101: 255-261.

Polge C. Embryo transplantation and preservation. In: Cole OJ, Foxcroft GR (eds.), Control of Pig Reproduction. Butterworth Scientific, London. 1982; 277-291.

Pope CE, Day BN. Development of embryos following restriction to the ampullar portion of the oviduct. J Reprod Fertil 1972; 31: 135-138.

Pope WF. Uterine asynchrony: a cause of embryonic loss. Biol Reprod 1988; 39: 999-1003.

Pope WF. Embryonic Mortality in Swine. In: Zavy MT, Geisert RD (eds.), Embryonic Mortality in Domestic Species. CRC Press. 1994; 53-77.

Pope WF, First NL. Factors affecting the survival of pig embryos. Theriogenology 1985; 23: 91-105.

Pope WF, Lawyer MS, Nara BS, First NL. Effect of asynchronous superinduction of embryo survival and range of blastocyst development in swine. Biol Reprod 1986; 35: 133-137.

Pope WF, Maurer RR, Stormshak F. Intrauterine migration of the porcine embryo: influence of estradiol-17beta and histamine. Biol Reprod 1982a; 27: 575-579.

Pope WF, Maurer RR, Stormshak F. Survival of porcine embryos after asynchronous transfer. Proc Soc Exp Biol Med 1982b; 171: 179-183.

Pope WF, Wilde MH, Xie S. Effect of electrocautery of nonovulated day 1 follicles on subsequent morphological variation among day 11 porcine embryos. Biol Reprod 1988; 39: 882-887.

Pope WF, Xie S, Broermann DM, Nephew KP. Causes and consequences of early embryonic diversity in the pig. J Reprod Fertil Suppl 1990; 40: 251-260.

Poretsky L, Chun B, Liu HC, Rosenwaks Z. Insulin-like growth factor II (IGF-II) inhibits insulin-like growth factor binding protein I (IGFBP-1) production in luteinized human granulosa cells with a potency similar to insulin-like growth factor I (IGF-I) and higher than insulin. J Clin Endocrinol Metab 1996; 81: 3412-3414.

Purvis J, Skelton JO, Quirk MN, Moore AB, Whitley NC, Cox NM. Influence of insulin and insulin-like growth factor-I (IGF-I) on the function of porcine ovarian follicles in culture. J Anim Sci 1997; 75(Suppl. 1): 1.

Pusateri AE, Rothschild MF, Warner CM, Ford SP. Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on day 9 to 13 of gestation. J Anim Sci 1990; 68: 3727-3735.

Pusateri AE, Smith JM, Smith JW 2nd, Thomford PJ, Diekman MA. Maternal recognition of pregnancy in swine. I. Minimal requirement for exogenous estradiol-17beta to induce either short or long pseudopregnancy in cycling gilts. Biol Reprod 1996; 55: 582-589.

Quesnel H, Pasquier A, Mounier AM, Prunier A. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J Anim Sci 1998; 76: 856-863.

Quesnel H, Prunier A. Endocrine bases of lactational anestrus in the sow. Reprod Nutr Dev 1995; 35: 395-414.

Ramirez-Godinez JA, Kiracofe GH, Schalles RR, Niswender GD. Endocrine patterns in the postpartum beef cow associated with weaning: a comparison of the short and subsequent normal cycles. J Anim Sci 1982; 55: 153-158.

Raposinho PD, Broqua P, Pierroz DD, Hayward A, Dumont Y, Quirion R, Junien JL, Aubert ML. Evidence that the inhibition of luteinizing hormone secretion exerted by central administration of neuropeptide Y (NPY) in the rat is predominantly mediated by the NPY-Y5 receptor subtype. Endocrinology 1999; 140: 4046-4055.

Rein MS, Schomberg DW. Characterization of insulin receptors on porcine granulosa cells. Biol Reprod 1982; 26 (Suppl. 1): 113.

Richards RG, Gadsby JE, Almond GW. Differential effects of LH and PGE2 on progesterone secretion by small and large porcine luteal cells. J Reprod Fertil 1994; 102: 27-34.

Roberts RM, Xie S, Trout WE. Embryo-uterine interactions in pigs during week 2 of pregnancy. J Reprod Fertil Suppl 1993; 48: 171-186.

Rodriguez M, Arias P, Refojo D, Feleder C, Moguilevsky J. Arrest of pulsatile luteinizing hormone (LH) secretion during insulin-induced hypoglycemia (IIH): improvement by intrahypothalamic perfusion with glucose. Exp Clin Endocrinol Diabetes 1999; 107: 257-261.

Rojanasthien SAM, Lundeheim N, Einarsson S. Luteinizing hormone response to different doses of synthetic gonadotropin releasing hormone during early and late lactation in primiparous sows. Anim Reprod Sci 1987; 13: 299-307.

Rojkittikhun T, Uvnas-Moberg, Einarsson S, Lundeheim N. Effects of weaning on plasma levels of prolactin, oxytocin, insulin, glucagon, glucose, gastrin and somatostatin in sows. Acta Physiol Scand 1991; 141: 295-303.

Ruiz de Galarreta CM, Fanjul LF, Hsueh AJ. Progestin regulation of progesterone biosynthetic enzymes in cultured rat granulosa cells. Steroids 1985; 46: 987-1002.

Sabatino FD, Collins P, McDonald JK. Neuropeptide Y stimulation of luteinizing hormone-releasing hormone secretion from the median eminence *in vitro* by estrogen-dependent and extracellular Ca2+-independent mechanisms. Endocrinology 1989; 124: 2089-2098.

Sabatino FD, Collins P, McDonald JK. Investigation of the effects of progesterone on neurpeptide Y-stimulated luteinizing hormone-releasing hormone secretion from the median eminence of ovariectomized and estrogen-treated rats. Neuroendocrinology 1990; 52: 600-607.

Sahu A, Crowley WR, Kalra SP. Evidence that hypothalamic neuropeptide Y gene expression increases before the onset of the preovulatory LH surge. J Neuroendocrinol 1995; 7: 291-296.

Sahu A, Kalra SP. Absence of increased neuropeptide Y neuronal activity before and during the luteinizing hormone (LH) surge may underlie the attenuated preovulatory LH surge in middle-aged rats. Endocrinology 1998; 139: 696-702.

Sahu A, Sininsky CA, Kalra PS, Kalra SP. Neuropeptide Y concentration in microdissected hypothalamic regions and *in vitro* release from the medial basal hypothalamus-preoptic area of streptozotocin-diabetic rats with and without insulin substitution therapy. Endocrinology 1990; 126:. 192-. 198.

Sahu A, Xu B, Kalra SP. Role of galanin (GAL) in stimulation of pituitary LH secretion as revealed by a specific receptor antagonist-galantide. Endocrinology 1994; 134: 529-539.

Saladin R, de Vos P, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J. Transient increase in obese gene expression after food intake or insulin administration. Nature 1995; 377: 527-529.

Samaras SE, Guthrie H, Barber J, Hammond J. Expression of the mRNAs for the insulin-like growth factors and their binding proteins during development of porcine ovarian follicles. Endocrinology 1993; 133: 3454-3468.

Samaras SE, Hagen DR, Bryan KA, Mondschein JS, Canning SF, Hammond JM. Effects of growth hormone and gonadotropin on the insulin-like growth factor system in the porcine ovary. Biol Reprod 1994; 50: 178-186.

Sauerwein H, Miyamoto A, Gunther J, Meyer HHd, Schams D. Binding and action of insulin-like growth factors and insulin in bovine luteal tissue during the oestrous cycle. J Reprod Fertil 1992; 96: 103-115.

Schams D, Kosmann M, Berisha B, Miyamoto A. Stimulation of vascular endothelial growth factor (VEGF) in cultured bovine granulosa-luteal cells. Biol Reprod 1999; 60 (Suppl. 1): 462.

Schneider JE, Friedenson DG, Hall AJ, Wade GN. Glucoprivation induces anestrus and lipoprivation may induce hibernation in Syrian hamsters. Am J Physiol 1993; 264: R573-R577.

Schneider JE, Goldman MD, Tang S, Bean B, Ji H, Friedman MI. Leptin indirectly affects estrous cycles by increasing metabolic fuel oxidation. Horm Behav 1998; 33: 217-228.

Schneider JE, Zhou D. Interactive effects of central leptin and peripheral fuel oxidation on estrous cyclicity. Am J Physiol 1999; 277: R1020-1024.

Schoham Z, Schachter M. Estrogen biosynthesis-regulation, action, remote effects, and value of monitoring in ovarian stimilation cycles. Fertil Steril 1996; 65: 687-701.

Schreihofer DA, Amica JA, Cameron JL. Reversal of fasting-induced suppression of luteinizing hormone (LH) secretion in male rhesus monkey by intragastric nutrition infusion: evidence for rapid stimulation of LH by nutritional signals. Endocrinology 1993; 132: 1890-1897.

Schreihofer DA, Renda F, Cameron JL. Feeding induced stimulation of luteinizing hormone secretion in male rhesus monkeys is not dependent on a rise in blood glucose concentration. Endocrinology 1996; 137: 3770-3776.

Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr, Woods SC, Seeley RJ, Weigle DS. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996a; 45: 531-535.

Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996b; 98: 1101-1106.

Schwartz MW, Dallman MF, Woods SC. Hypothalamic response to starvation: Implications for the study of wasting disorders. Am J Physiol 1995; 269: R949-R957.

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte JrD. Insulin in the brain: A hormonal regulator of energy balance. Endocr Rev 1992a; 13: 387-414.

Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Schewrink A, Kahn SE, Baskin DG, Woods SC, Figlewicz DR, Porte D Jr. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology 1992b; 130: 3608-3616.

Schwartz MW, Figlewicz DP, Kahn SE, Baskin D, Porte JrD, Greenwood MRC. Insulin binding to brain capillaries is reduced in genetically obese hyperinsulinemic Zucker rats. Peptides 1990; 11: 467-472.

Schwartz MW, Seeley RJ. The new biology of body weight regulation. J Am Diet Assoc 1997; 97: 54-58.

Scofield AM, Clegg FG, Lamming GE. Embryonic mortality and uterine infection in the pig. J Reprod Fertil 1974; 36: 353-361.

Sesti LAC, Britt JH. Agonist-inducing release of gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone and their association with basal secretion of luteinizing hormone and follicle-stimulating hormone throughout lactation in sows. Biol Reprod 1993a; 49: 332-339.

Sesti LAC, Britt JH. Relationship of secretion of GnRH *in vitro* to changes in pituitary concentrations of LH and FSH and serum concentrations of LH during lactation in sow. J Reprod Fertil 1993b; 98: 393-400.

Sesti LAC, Britt JH. Secretion of gonadotropins and estimated releasable pools of gonadotropin-releasing hormone and gonadotropins during establishment of suckling-induced inhibition of gonadotropin secretion in the sow. Biol Reprod 1994; 50: 1078-1086.

Shaw HJ. Control of ovarian function in lactating and weaned sows. Sutton Bonington, Loughborough, UK: University of Nottingham. 1984. Ph.D. Thesis.

Shaw HJ, Foxcroft GR. Relationship between LH, FSH and prolactin and reproductive activity in the weaned sow. J Reprod Fertil 1985; 75: 17-28.

Shimasaki S, Shimonaka M, Ui M, inoue S, Shibata F, Ling N. Structural characterization of a follicle-stimulating hormone action inhibitor in porcine ovarian follicular fluid. Its identification as the insulin-like growth factor-binding protein. J Biol Chem 1990; 265: 2198-2202.

Shukovski L, Findlay J, Robertson D. The effect of follicle-stimulating hormone-suppressing protein or follistatin on luteinizing bovine granulosa cells *in vitro* and its antagonistic effect on the action of activin. Endocrinology 1991; 126: 3395-3402.

Simpson ER, McCarthy JL, Peterson JA. Evidence that the cycloheximide-sesitive site of adrenocorticotropic hormone action is in the mitochondrion. J Biol Chem 1978; 253: 3135-3139.

Sites CK, Patterson K, Jamison CS, Degen SJF, Labarbera AR. Follicle-stimulating hormone (FSH) increases FSH receptor message ribinucleis acid while decreasing FSH binding in cultured porcine granulosa cells. Endocrinology 1994; 134: 411-417.

Smith CA, Almond GW, Dial GD. Changes in luteinizing hormone concentrations after abortion, parturition, and weaning in primiparous sows. Anim Reprod Sci 1992; 27: 169-182.

Smith MF. Recent advances in corpus luteum physiology. J Dairy Sci 1986; 69: 911-926.

Smith MF, Kemper CN, Smith GW, Goetz TL, Jarrell VL. Production of tissue inhibitor of metalloproteinases-1 by porcine follicular and luteal cells. J Anim Sci 1994a; 72: 1004-1012.

Smith MF, McIntush EW, Smith GW. Mechanisms associated with corpus luteum development. J Anim Sci 1994b; 72: 1857-1872.

Soede NM, Noordhuizen JPTM, Kemp B. The duration of ovulation in pig, studied by transrectal ultrasonography, is not related to early embryonic diversity. Theriogenology 1992; 38: 653-666.

Spicer LJ, Stewart RE, Alvarez P, Francisco CC, Keefer BE. Insulin-like growth factor-binding protein-2 and -3: Their biological effects in bovine thecal cells. Biol Reprod 1997; 56: 1458-1465.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995; 377: 530-532.

Stevenson JS, Britt JH. Luteinizing hormone, total estrogens and progesterone secretion during lactation and after weaning in sows. Theriogenology 1981; 14: 453-462.

Stevenson JS, Cox NM, Britt JH. Role of the ovary in controlling luteinizing hormone, follicle stimulating hormone and prolactin secretion during and after lactation in pigs. Biol Reprod 1981; 24: 341-353.

Stocco DM. The steroidogenic acute regulatory (StAR) protein two years later. An update. Endocrine 1997; 6: 99-109.

Stocco DM. Recent advances in the role of StAR. Rev Reprod 1998; 3: 82-85.

Stouffer RL, Aladin Chandrasekher Y, Slayden OD, Zelinski-Wooten MB. Gonadorphic and local control of the developing corpus luteum in rhesus monkeys. Hum Reprod 1993; 8 (Suppl.2): 107-111.

Strack AM, Sebastian RJ, Schwartz MW, Dallman MF. Glucocorticoids and insulin: reciprocal signals for energy balance. Am J Physiol 1995; 268: R142-R149.

Stroband HWJ, Taverne N, Van der Bogaard M. The pig blastocyst: its ultrastructure and the uptake of protein macromolecules. Cell Tissue Res 1984; 235: 347-356.

Stroband HWJ, van der Lende T. Embryonic and uterine development during early pregnancy in pigs. J Reprod Fertil Suppl 1990; 40: 261-277.

Strott CA, Cargille CM, Ross GT, Lipsett MB. The short luteal phase. J Clin Endocrinol Metab 1970; 30: 246-251.

Sugino K, Kurosawa N, Nakamura T, Takio K, Shimasaki S, Ling N, Titani K, Sugino H. Molecular heterogeneity of follistatin, an activin-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. J Biol Chem 1993; 268: 15579-15587.

Sutton S, Toyama T, Otto S, Plotsky P. Evidence that neuropeptide Y (NPY) release into the hypophyseal-portal circulation participates in priming gonadotropes to the effects of gonadotropin releasing hormone (GnRH). Endocrinology 1988; 123: 1208-1210.

Swerdloff RS, Batt RA, Bray GA. Reproductive hormonal function in the genetically obese (ob/ob) mouse. Endocrinology 1976; 98: 1359-1364.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI.. Identification and expression cloning of a leptin receptor, OB-R. Cell 1995; 83: 1263-1271.

Taya K, Kaneko H, Watanabe G, Sasamoto S. Inhibin and secretion of FSH in estrus cycles of cows and pigs. J Reprod Fertil Suppl 1991; 43: 151-162.

Thompson MP. Meal-feeding specifically induces obese mRNA expression. Biochem Biophys Res Commun 1996; 224: 332-337.

Toaff ME, Strauss JF, III, Flickinger GL, Shattil SL. Relationship of cholesterol supply to luteal mitochondrial steroid synthesis. J Biol Chem 1979; 254: 3977-3982.

Tokach M, Pettigrew J, Dial G, Wheaton J, Crooker B, Johnson L. Characterization of luteinizing hormone secretion in the primiparous, lactating sow: relationship to blood metabolites and return-to-estrus interval. J Anim Sci 1992a; 70: 2. 195-2220.

Tokach M, Pettigrew J, Dial G, Wheaton J, Crooker B, Koketsu Y. Influence of glucose infusions on luteinizing hormone secretion in the energy-restricted, primiparous, lactating sow. J Anim Sci 1992b; 70: 2202-2209.

Tonetta SA. Paracrine control of 3 beta-hydroxysteroid dehydrogenase, 17,20-lyase and aromatase enzyme systems in porcine thecal cells. Adv. Exp. Med. Biol. 1987; 219: 665-670.

Tonetta SA, diZerega GS. Local regulatory factors controlling folliculogenesis in pigs. J Reprod Fertil Suppl 1990; 40: 151-161.

Trout WE, Killen JH, Christenson RK, Schanbacher BD, Ford JJ. Effects of weaning on concentrations of inhibin in follicular fluid and plasma of sows. J Reprod Fertil 1992; 94:107-114.

Tsukahara S, Tsukamura H, Foster DL, Maeda KI. Effect of corticotropin-releasing hormone antagonist on oestrogen-dependent glucoprivic suppression of luteinizing hormone secretion in female rats. J Neuroendocrinol 1999; 11: 101-105.

Tuls J, Geren L, Lambeth JD, Millett F. The use of a specific fluorescence probe to study the interaction of adrenodoxin with adrenodoxin reductase and cytochrome P450scc. J Biol Chem 1987; 262: 10020-10025.

Urban JH, Das I, Levine JE. Steroid modulation of neuropeptide Y-induced luteinizing hormone releasing hormone release from median eminence fragments from male rats. Neuroendocrinology 1996; 63: 112-1. 19.

Urban R, Garmey J, Shupnik M, Veldhuis J. Insulin-like growth factor type 1 increases concentrations of messenger ribonucleic acid encoding cytochrome P450 cholesterol side chain cleavage enzyme in primary cultures of porcine granulosa cells. Endocrinology 1990; 127: 2481-2488.

Urban RJ, Shupnik MA, Bodenburg YH. Insulin-like growth factor-I increases expression of the porcine P-450 cholesterol side chain cleavage gene through a GC-rich domain. J Biol Chem 1994; 269: 25761-25769.

Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 1986; 321: 776-779.

van den Brand H, Dieleman SJ, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: I. Effects on glucose, insulin, and luteinizing hormone and on follicle development, weaning-to-estrus interval, and ovulation rate. J Anim Sci 2000a; 78: 396-404.

van den Brand H, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: II. Effects on periestrus hormone profiles and embryonal survival. J Anim Sci 2000b; 78: 405-411.

Van der Lende T, Schoenmaker GJW. The relationship between ovulation rate and litter size before and after day 35 of pregnancy in gilts and sows: an analysis of published data. Livest Prod Sci 1990; 26: 217-229.

Van der Lende T, Soede NM, Kemp BV. Embryo mortality and prolificacy in the pig. In: Cole DA, Wiseman J, Varley MA (eds.), Principles of Pig Science. Nottingham: Nottingham University Press. 1994; 297-317.

Van der Meulen J, Te Kronnie G, Van Deursen R, Geelen J. Aromatase activity in individual Day-11 pig blastocysts. J Reprod Fertil 1989; 87: 783-788.

Vanhatalo S, Soinila S. Pituitary gland receives both central and peripheral neuropeptide Y innervation. Brain Res 1996; 740: 253-260.

Varley MA, Foxcroft GR. Endocrinology of the lactating and weaned sow. J Reprod Fertil Suppl 1990; 40: 47-61.

Vega M, Castillo T, Retamales I, Las Heras J, Devoto L, Videla LA. Steroidogenic capacity and oxidative stress-related parameters in human luteal cell regression. Free Rad Biol Med 1994; 17: 493-499.

Vega M, Devoto L. Autocrine/paracrine regulation of normal human corpus luteum development. Sem Reprod Endocrinol 1997; 15: 353-362.

Veldhuis JD, Furlanetto RW. Tropic actions of human somatomedin C/insulin-like growth factor 1 on ovarian cells: *in vitro* studies with granulosa cells. Endocrinology 1985; 116: 1235-1242.

Veldhuis JD, Nestler JE, Strauss JF 3rd. The insulin-like growth factor, somatomedin-C, modulates low density lipoprotein metabolism by swine granulosa cells. Endocrinology 1987; 121: 340-346.

Vesseur PC. Causes and consequences of variation in weaning to estrus interval in the sow. Rosmalen, NETH: Research Institute for Pig Husbandary: 1997. Ph.D. Thesis.

Wade GN, Schneider JE, Li H. Control of fertility by metabolic cues. Am J Physiol 1996; 270: E1-E19.

Wandji SA, Srsen V, Voss AK, Eppig JJ, Fortune JE. Initiation *in vitro* of growth of bovine primordial follicles. Biol Reprod 1996; 55: 942-948.

Watanabe H, Takebe K. Evidence that neuropeptide Y secretion in the median eminence increases prior to the liteinizing hormone surge in ovariectomized steroid-primed rats: estimation by push-pull perfusion. Neurosci Lett 1992; 146: 57-59.

Watson J, Patek CE. Steroid and prostaglandin secretion by the corpus luteum, endometrium and embryos of cyclic and pregnant pigs. J Endocrinol 1979; 82: 425-428.

Wehrenberg WB, Corder R, Gaillard RC. A physiological role for neuropeptide Y in regulating the estrogen/progesterone induced luteinizing hormone surge in ovariectomized rats. Neuroendocrinology 1989; 49: 680-682.

Whitley NC, Moore AB, Cox NM. Comparative effects of insulin and porcine somatotropin on postweaning follicular development in primiparous sows. J Anim Sci 1998a; 76: 1455-1462.

Whitley NC, Thomas MNQ, Skelton JO, Moore AB, Purvis J, Qiu Y, Cox NM. Influence of insulin on follicular development and the intrafollicular insulin-like growth factor I (IGF-I) system in sows after weaning. J Reprod Fertil 1998b; 112: 175-184.

Wiesak T, Hunter MG, Foxcroft GR. Effect of prostaglandins on luteal function during early pregnancy in pigs. J Reprod Fertil 1992; 95: 831-840.

Wilde MH, Xie S, Day ML, Pope WF. Survival of small and large littermate blastocysts in swine after synchronous and asynchronous transfer procedures. Theriogenology 1988; 30: 1069-1074.

Wilks JW, Hodgen GD, Ross GT. Luteal phase defects in the rhesus monkey: the significance of serum FSH: LH ratios. J Clin Endocrinol Metab 1976; 43: 1261-1267.

Williams N, Lancas M, Cameron J. Stimulation of luteinizing hormone secretion by food intake: evidence against a role for insulin. Endocrinology 1996; 137: 2565-2571.

Willis D, Franks S. Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. J Clin Endocrinol Metab 1995; 80: 3788-3790.

Willis DS, Mason HD, Watson H, Franks S. Developmentally regulated responses of human granulosa cells to insulin-like growth factors (IGFs): IGF-I and IGF-II action mediated via the type-I IGF receptor. J Clin Endocrinol Metab 1998; 83: 1256-1259.

Winters TA, Hanten JA, Veldhuis JD. In situ amplification of the cytochrome P-450 cholesterol side-chain cleavage enzyme mRNA in single porcine granulosa cells by IGF-1 and FSH acting alone or in concert. Endocrine 1998; 9: 57-63.

Woller MJ, McDonald JK, Reboussin DM, Terasawa E. Neuropeptide Y is a neuromodulator of pulsatile luteinizing hormone-releasing hormone release in the gonadectomized rhesus monkey. Endocrinology 1992; 130: 2333-2342.

Woodruff TK, Mather JP. Inhibin, activin and the female reproductive axis. Annu Rev Physiol 1995; 57: 219-244.

Wu MC, Shin WJ, Dziuk PJ. Influence of pig embryos on uterine growth. J Anim Sci 1988; 66: 1721-1726.

Wu TJ, McArthur NH, Harms PG. A possible dual mechanism involved in the inhibitory influence of suckling on luteinizing hormone release in the ovariectomized rat. Biol Reprod 1992; 46: 653-657.

Wuttke W, Pitzel L, Knoke I, Theiling K, Jarry H. Immune-endocrine interactions affecting luteal function in pigs. J Reprod Fertil Suppl 1997; 52: 19-29.

Xiao S, Findlay J. Interactions between activin and FSH suppressing protein and their mechanisms of action on cultured rat granulosa cells. Mol Cell Endocrinol 1991; 79: 99-107.

Xiao S, Robertson DM, Findlay JK. Effects of activin and follicle-stimulating hormone(FSH)-suppressing protein/follistatin on FSH receptors and differentiation of cultured rat granulosa cells. Endocrinology 1992; 131: 1009-1016.

Xie S, Broermann DM, Nephew KP, Bishop MD, Pope WF. Relationship between oocyte maturation and fertilization on zygotic diversity in swine. J Anim Sci 1990a; 68: 2027-2033

Xie S, Broermann DM, Nephew KP, Geisert RD, Pope WF. Ovulation and early embryogenesis in swine. Biol Reprod 1990b; 43: 236-240.

Xu B, Sahu A, Crowley WR, Leranth C, Horvath T, Kalra SP. Role of neuropeptide Y in episodic luteinizing hormone release in ovariectomized rats: an excitatory component and opioid involvement. Endocrinology 1993; 133: 747-754.

Xu G, Sahu A, Kalra PS, Crowley WR, Kalra SP. Disinhibition of opioid influence augments hypothalamic neuropeptide Y expression and pituitary LH release: effects of NPY mRNA antisense oligodeoxynucleotides. Endocrinology 1996; 137: 78-84.

Xu YP, Chedrese PJ, Thacker PA. Growth hormone as an amplifier of insulin-like growth factor-1 action: potentiated oestradiol accumulation. J Endocrinol 1997; 152: 201-209.

Xue JL, Dial GD, Marsh WE, Davies PR, Momont HW. Influence of lactation length on sow productivity. Livest Prod Sci 1993; 34: 253-265.

Yamamoto S, Konishi I, Tsuruta Y, Nanbu K, Mandai M, Kuroda H, Matsushita K, Hamid AA, Yura Y, Mori T. Expression of vascular endothelial growth factor (VEGF) during folliculogenesis and corpus luteum formation in the human ovary. Gynecol Endocrinol 1997; 11: 371-381.

Yan Z, Neulen J, Raczek S, Weich HA, Keck C, Grunwald K, Breckwoldt M. Vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) production by luteinized human granulosa cells *in vitro*, a paracrine signal in corpus luteum formation. Gynecol Endocrinol 1998; 12: 149-153.

Yan Z, Weich HA, Bernart W, Breckwoldt M, Neulen J. Vascular endothelial growth factor (VEGF) messenger ribonucleic acid (mRNA) expression in luteinized human granulosa cells *in vitro*. J Clin Endocrinol Metab 1993; 77: 1723-1725.

Yang H. Impact of dietary lysine during lactation on lactation and subsequent reproductive performance of sows. USA: University of Minnesota. 1998; Ph.D. Thesis.

Yoshimura Y. Insulin-like growth factors and ovarian physiology. J Obstet Gynaecol Res 1998; 24: 305-323.

Yoshimura Y, Aoki N, Sueoka K, Miyazaki T, Kuji N, Tanaka M, Kobayashi T. Interactions between insulin-like growth factor-I (IGF-I) and the renin-angiotensin system in follicular growth and ovulation. J Clin Invest 1996; 98: 308-316.

Youngs CR, Ford SP, McGinnis LK, Anderson LH. Investigations into the control of litter size in swine: 1. Comparative studies on *in vitro* development of Meishan and Yorkshire preimplantation embryos. J Anim Sci 1993; 71: 1561-1565.

Yuan W, Connor ML, Buhr MM. Responsiveness of porcine large and small luteal cells to luteotropic or luteolytic hormones and cell morphologic changes during the estrous cycle and pregnancy. J Anim Sci 1993; 71: 481-491.

Yuan W, Lucy MC, Smith MF. Messenger ribonucleic acid for insulin-like growth factors-I and -II, insulin-like growth factor-binding protein-2, gonadotropin receptors, and steroidogenic enzymes in porcine follicles. Biol Reprod 1996; 55: 1045-1054.

Yuan W, Lucy MC. Effects of growth hormone, prolactin, insulin-like growth factors, and gonadotropins on progesterone secretion by porcine luteal cells. J Anim Sci 1996; 74: 866-672.

Zak LJ, Cosgrove JR, Aherne FX, Foxcroft GR. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J Anim Sci 1997a; 75: 208-216.

Zak LJ, Xu X, Hardin RT, Foxcroft GR. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J Reprod Fertil 1997b; 110: 99-106.

Zak LJ, Williams IH, Foxcroft GR, Pluske JR, Cegielski AC, Clowes EJ, Aherne FX. Feeding lactating primiparous sows to establish three divergent metabolic states: I. Associated endocrine changes and postweaning reproductive performance. J Anim Sci 1998; 76: 1145-1153.

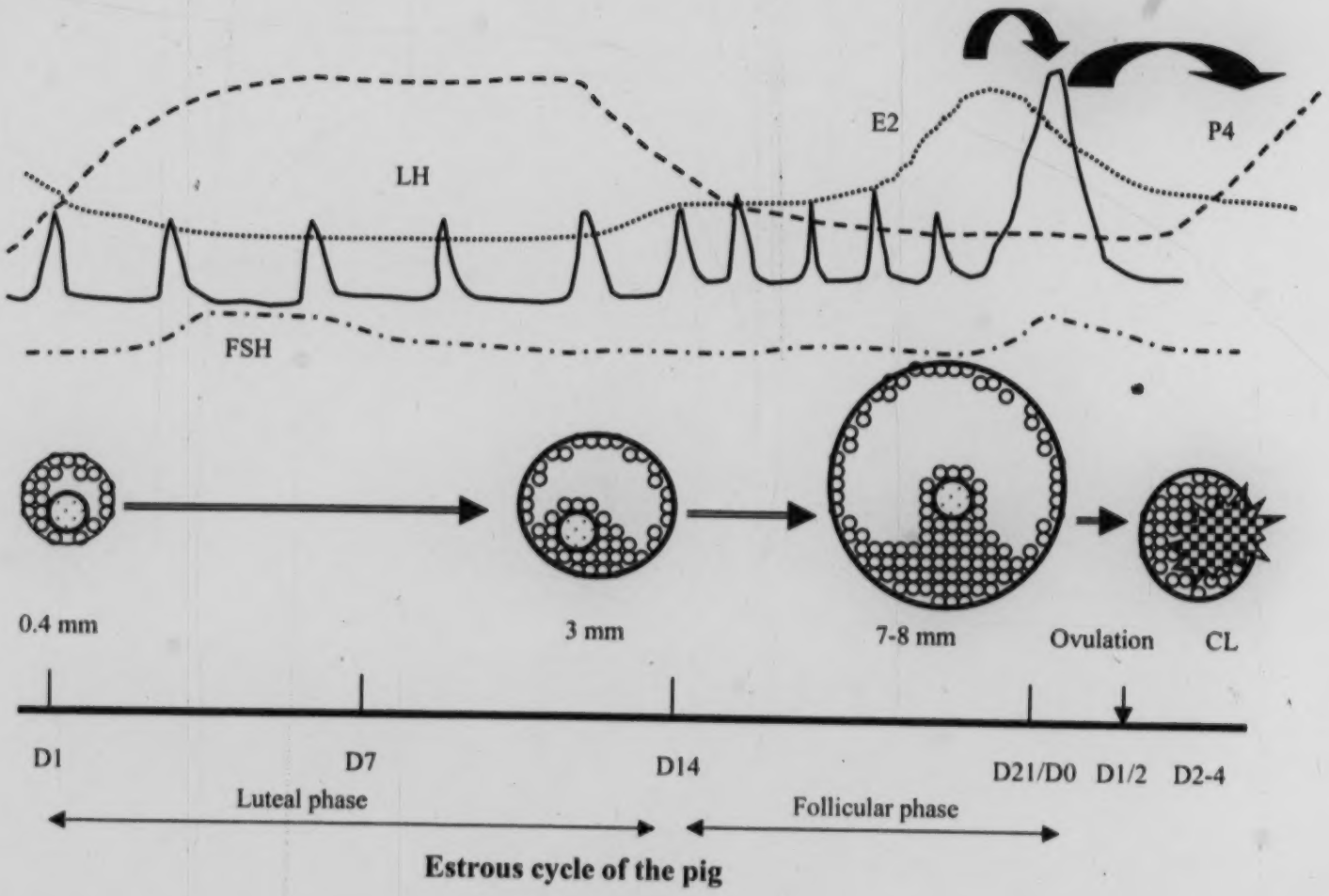
Zamorano PL, Mahesh VB, de Sevilla LM, Chorich LP, Bhat GK, Brann DW. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissue of the rat. Neuroendocrinology 1997; 65: 223-228.

Zhiwen Z, Herington, Carson R, Findlay J, Burger H. Direct inhibition of rat granulosa cell inhibin production by epidermal growth factor. Mol Cell Endocrinol 1987; 54: 213-220.

Zhou J, Adesanya OO, Vatzias G, Hammond JM, Bondy CA. Selective expression of insulin-like growth factor system components during ovary folliclular selection. Endocrinology 1996; 137: 4893-4901.

Figure 2.1. Estrous cycle of the pig

The length of the estrous cycle of pigs is about 21 days (D) and is divided into two phases, the luteal phase and follicular phase. During each estrous cycle, both FSH and LH stimulate follicles to grow from small follicles into large pre-ovulatory follicles. As these follicles grow, they secrete increasing amount of estradiol. Plasma estradiol (E_2) levels peak at estrus and induce preovulatory LH surge and in turn ovulation. After ovulation, follicular cells re-differentiate to form the corpus luteum (CL) and secrete progesterone (P_4), which is essential for the maintenance of pregnancy. Therefore, the preovulatory LH surge is indirectly responsible for the rise of progesterone after ovulation.



Estrous cycle of the pig

Figure 2.2. Three endocrine phases of lactation and follicle development in the lactating sow (Modified after Britt, 1996)

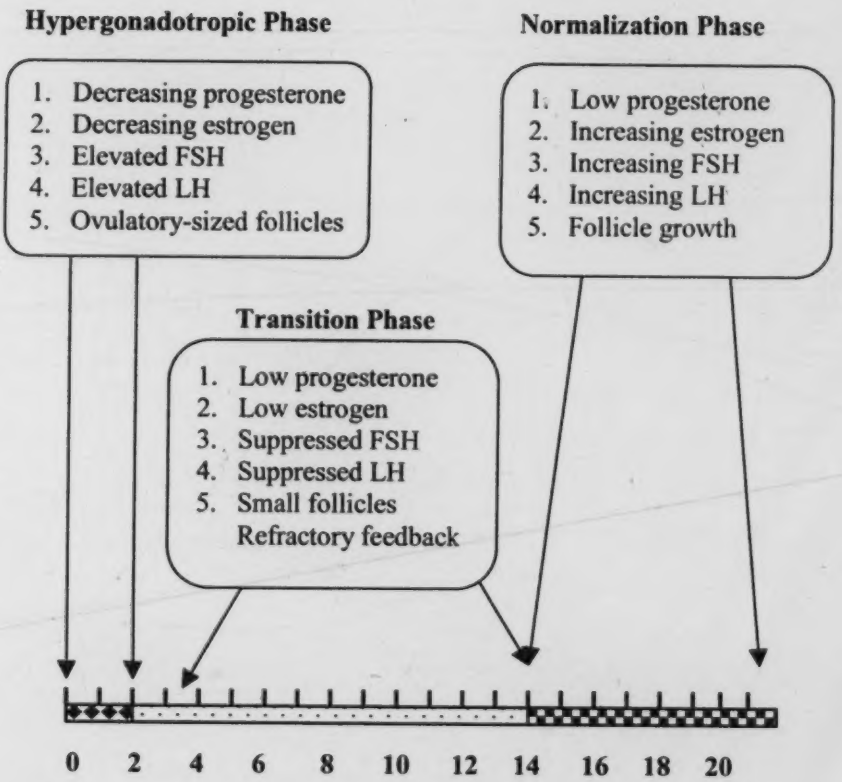

Hypergonadotropic Phase
1. Decreasing progesterone
2. Decreasing estrogen
3. Elevated FSH
4. Elevated LH
5. Ovulatory-sized follicles
Normalization Phase
1. Low progesterone
2. Increasing estrogen
3. Increasing FSH
4. Increasing LH
5. Follicle growth
Transition Phase
1. Low progesterone
2. Low estrogen
3. Suppressed FSH
4. Suppressed LH
5. Small follicles
Refractory feedback
0 2 4 6 8 10 12 14 16 18 20
Stage of lactation (days)

Figure 2.3. Progesterone synthesis pathways within the luteal cell (see text for details).

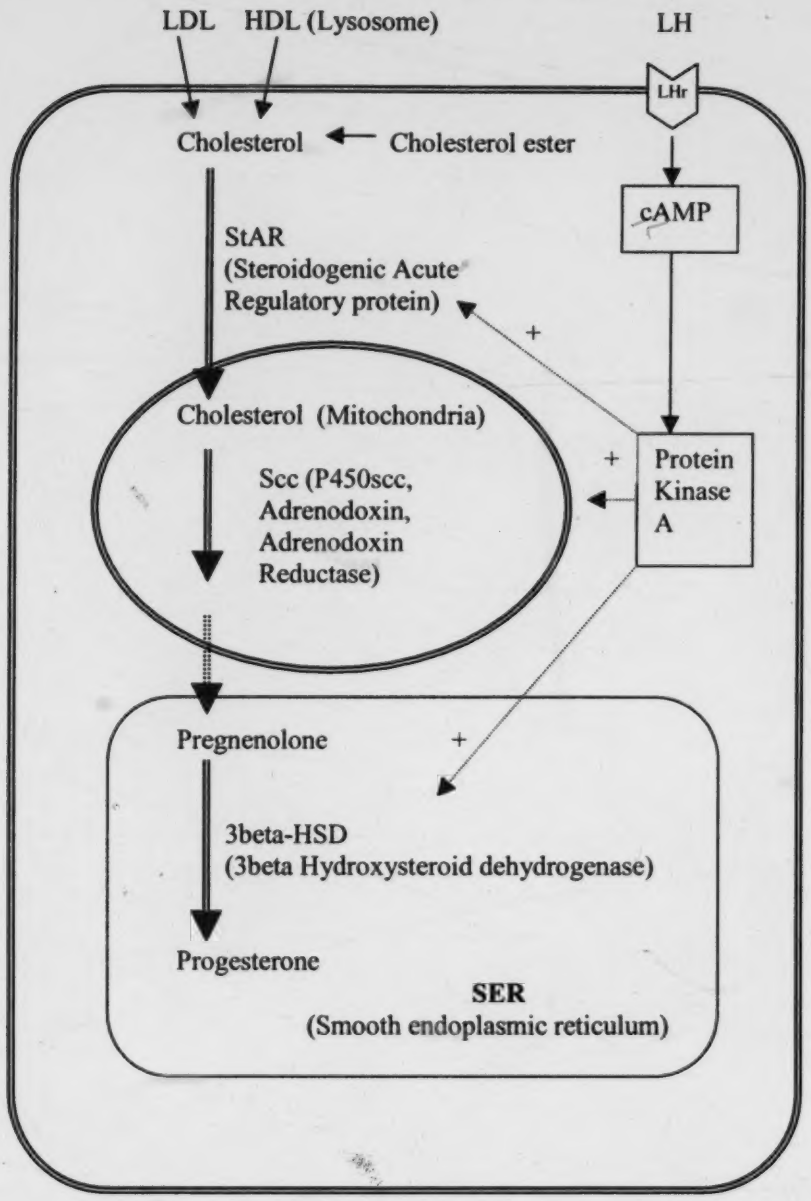
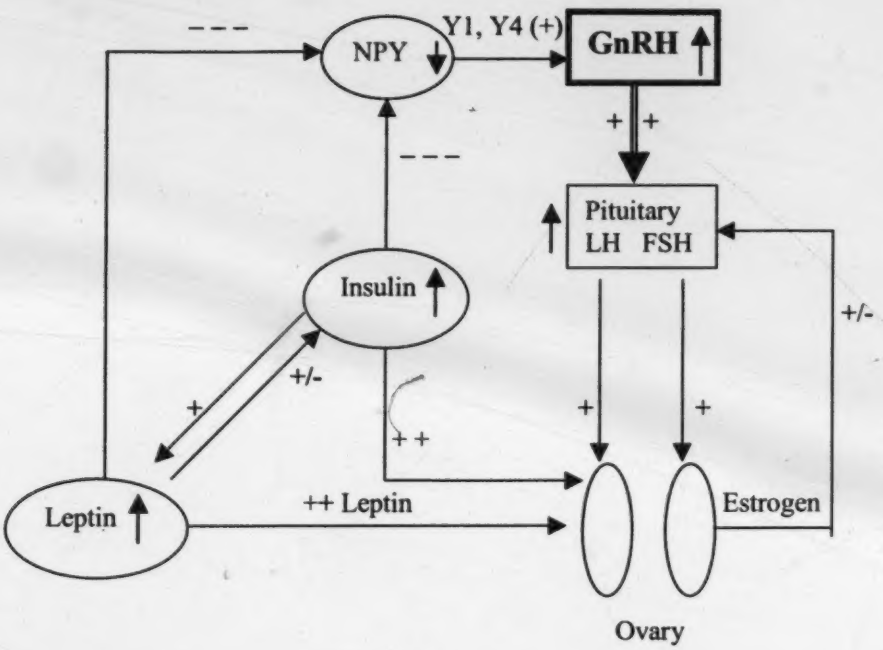

LDL
HDL (Lysosome)
LH
LHr
Cholesterol
Cholesterol ester
cAMP
StAR
(Steroidogenic Acute
Regulatory protein)
+
Cholesterol (Mitochondria)
Scc (P450scc,
Adrenodoxin,
Adrenodoxin
Reductase)
+
Protein
Kinase
A
Pregnenolone
+
3beta-HSD
(3beta Hydroxysteroid dehydrogenase)
Progesterone
SER
(Smooth endoplasmic reticulum)

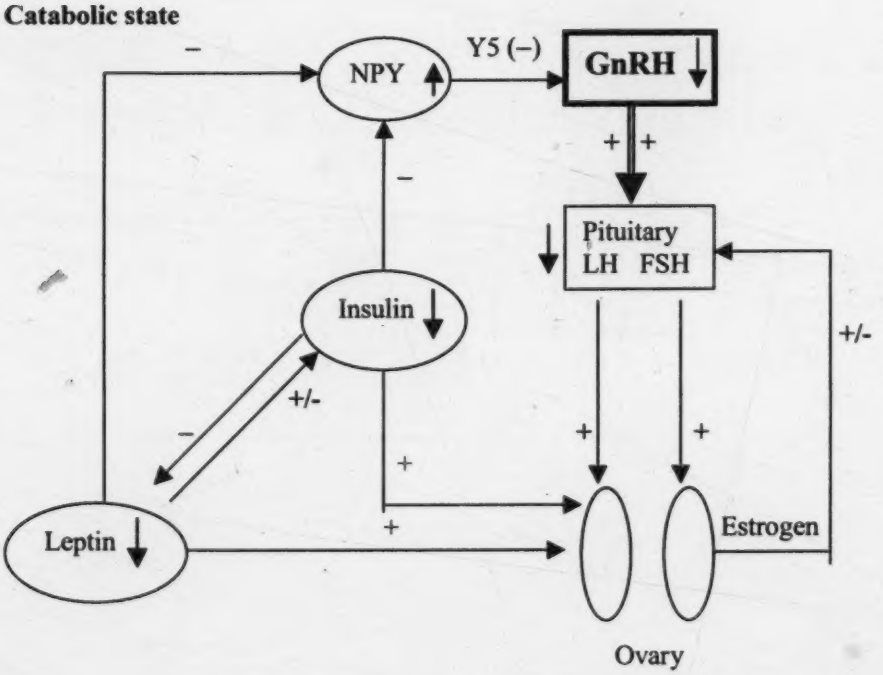
Figure 2.4. The relationships between insulin, leptin, NPY, pituitary gonadotropins, and ovarian function in anabolic and catabolic states.

Possible mechanisms mediating nutritional effects on GnRH and gonadotropin secretion are in part achieved by changes in insulin and leptin concentrations. In normal fed animals **(A)**, high insulin concentrations stimulate leptin secretion. Both insulin and leptin very actively inhibit NPY secretion. Low, intermittent NPY production acts via Y1 and Y4 receptors to induce GnRH secretion. In feed-restricted animals **(B)**, insulin and leptin concentrations are depressed and NPY levels are increased. High, continuous NPY production acts negatively via Y5 receptors to inhibit GnRH secretion.

A. Anabolic state



B. Catabolic state



CHAPTER 3

PROGESTERONE THERAPY DURING EARLY PREGNANCY AND EMBRYONIC SURVIVAL IN PRIMIPAROUS WEANED SOWS

INTRODUCTION

Embryonic mortality in swine represents a major loss of productivity in that some 30% of eggs ovulated fail to materialize as live born piglets. Attempts to improve embryo survival in the pig have been largely unsuccessful and the mechanisms mediating effects on embryo survival have not been clearly identified. Primiparous weaned sow models were established in our laboratory in which embryo survival could be manipulated. Zak et al. (1997) showed that when primiparous sows were fed to appetite from farrowing to d 21, but then restricted to 50% of ad libitum intake from d 22 to 28 of lactation, subsequent embryonic survival at d 28 of gestation decreased to 64.4%, compared to 87.5% in sows fed to appetite throughout the lactation period.

Nutritional effects on embryonic loss can be attributed to several factors (Foxcroft, 1997). High feed intakes immediately after mating have been associated with reduced peripheral progesterone concentrations, possibly through changes in metabolic clearance rates, and increased embryonic mortality (Den Hartog et al., 1981; Dyck and Strain 1983; Kirkwood and Thacker, 1988; Parr et al., 1993; Jindal et al., 1996). Progesterone concentrations during early pregnancy in gilts were positively associated with embryonic survival (Ashworth et al. 1989; Pharazyn et al. 1991; Jindal, 1996) and a similar association was also established in the primiparous weaned sow (Clowes, 1993; Jindal, 1996; Zak et al., 1998; van den Brand et al., 2000). Collectively, these studies suggest that changes in plasma progesterone may mediate nutritional effects on embryonic survival. Direct evidence to support this hypothesis came from experiments in which exogenous progesterone treatment reversed detrimental effects of high plane feeding on embryo survival in sheep (Parr et al. 1987; Ashworth et al. 1989). Also in gilts with high ovulation rates but low embryonic survival as a consequence of ad libitum feeding before and after mating, supplementary

---

*: A paper based on Chapter 3 has been published in the Journal of Animal Science (1998, 76:1922-1928) by Mao J and Foxcroft GR.

progesterone after mating increased embryonic survival at d 30 from 66.4 to 82.8% (Ashworth, 1991) and at d 28 from 70.0 to 84.4% (Jindal et al. 1997). Therefore, taking advantage of the experimental paradigm developed by Zak et al. (1997), the present study was undertaken 1) to determine whether negative effects of lactational feed restriction on embryonic survival in the primiparous weaned sow could be abrogated by administration of progesterone in early pregnancy, and 2) to confirm the functional relationship between progesterone in the post-ovulatory period and embryonic survival in primiparous lactating and weaned sows.

MATERIALS AND METHODS

Thirty-four lactating primiparous Camborough sows (Pig Improvement (Canada) Ltd.) were kept in barns with a totally controlled environment and used in a replicated experimental design, involving four farrowing groups. Whenever possible, the number of piglets per litter was standardized to 9 (the mean litter size) within 48 h after farrowing, resulting in 8.9 ± 0.1 and 8.7 ± 0.1 piglets suckling per litter for the control and progesterone group, respectively. All sows were fed ad libitum from farrowing until d 21 of lactation (farrowing day is d 0) a barley-soybean diet formulated (Table 3.1) to provide 3410 Kcal of DE/kg, 16.05% crude protein and 0.87% lysine to meet NRC requirements (1988). Feed allowance was then reduced to 50% of ad libitum intakes between d 22 of lactation and weaning at d 28, equally divided between meals at 0730 and 1500 h daily. Feed consumption was determined by the weigh-back method. Water was available ad libitum to both the sows and piglets throughout the experimental period. Sow body weight, backfat thickness at P_2, and litter weight were recorded at farrowing, d 21 and weaning.

From weaning, all sows were fed ad libitum with a barley-soybean diet formulated to provide 3045 Kcal of DE/kg, 13.89% crude protein and 0.60% lysine (Table 3.1) until slaughter. Onset of standing heat was detected twice daily at 0730 and 1930 using back pressure test during direct exposure to mature vasectomized boars for 15 minutes to determine weaning-to-estrus interval (WEI); mating was not permitted. The 24-h period after first detection of estrus was designated as d 0 of gestation. All experimental sows were artificially inseminated 12 and 24 h after the first observed

standing heat with pooled semen (3×10^9 spermatozoa/dose) from the same group of three fertile boars specifically designated for this experiment. Semen was provided by the Alberta Swine Genetics Corporation, Leduc, Alberta and was used for insemination up to 4 days after collection. All sows were inseminated by the same trained person.

Progesterone treatment

Within each replicate, sows were equally allocated in a stratified design according to percentage of body weight loss over the first 21 d of lactation to be treated with ethyl oleate (control group; n = 17) or to receive progesterone therapy (progesterone group, 25 mg P_4/ml in ethyl oleate; n = 17). All sows received a series of six intramuscular injections of progesterone or ethyl oleate immediately posterior to the ears at 12-h intervals, beginning 36 h after onset of standing heat. Based on the progesterone therapy schedule used in a previous gilt study (Jindal et al., 1997), the progesterone dose was set at 2 mg/kg body weight $^{0.75}$ calculated on an individual sow weight, based on body weight increment within a 10 kg range.

Blood sampling for progesterone assessment

On the basis of earlier data (Pharazyn, 1992; Jindal et al., 1996), blood samples (2.5 ml) were collected from a peripheral ear vein into heparinized tubes at 36, 48, 72 and 96 h after first detection of standing estrus during a brief period of nose-snare restraint. Plasma was harvested by centrifugation at 1,500 × g for 15 min immediately after collection and stored at -30°C until analysis for progesterone concentration using a Coat-A-Count progesterone kit (Diagnostic Products Corporation, Los Angeles, USA; Catalog # : TKPG1) validated for use as a direct assay with porcine plasma. No significant deviation from parallelism was apparent from assaying 100, 50, 25, 12.5 and 6.25 µl of a standard plasma pool. The sensitivity of the assay, defined as the apparent concentration two standard deviations (SD) below the counts at maximum binding, was 0.009 ng/tube (0.09 ng/ml). The intra-assay CV was 4.2%, and the inter-assay CV was 13.9%. Known amounts of unlabelled hormone added to a pool of porcine plasma were run with each assay and estimates of P4 measured averaged 105 ± 3.1% of hormone added.

Ovulation rate and embryonic survival

All sows were slaughtered at local abattoir on day 28 ± 3 of pregnancy (between d 23.5 and 29) without any bias for treatment groups (see Table 3.4). Immediately after slaughter, the reproductive tracts were recovered from each sow and both ovaries were examined to determine the number of corpora lutea (as the measure of ovulation rate). Several incisions into the ovarian tissue were made to determine the presence of any hidden corpora lutea. Each uterine horn was opened by blunt dissection along the antimesometrial axis and gentle eversion of the uterus was used to recover embryos within their trophoblastic vesicles. To provide an objective measure of abnormal development, embryos were classified as being nonviable on the basis of a crown-rump length of more than 2 SD less than the mean for embryos recovered from that sow (Jindal et al., 1996). Two measures of embryonic survival were then determined: 1) total embryonic survival, expressed as the percentage of corpora lutea represented by any embryo, and 2) viable embryonic survival, expressed as the percentage of corpora lutea represented by normally developed embryos.

Statistical analyses

Differences between control and progesterone groups for sow body weight, body weight change, sow backfat thickness at P_2, backfat thickness change, litter weight, piglet growth rate and weaning-to-estrus interval were analyzed using the SAS-GLM procedure (1990). The effect of progesterone treatment on ovulation rate, total number of embryos, total embryonic survival rate, number of viable embryos, viable embryonic survival rate, and plasma progesterone concentrations were also assessed using the GLM procedure of SAS (1990). For all these variables, replicate, treatment and the treatment by replicate interaction were included in the model, but no interaction between treatment and replicate was established. Sow was the experimental unit and was used as the estimate of error. The average crown-rump length of all embryos from each sow was analyzed, fitting day of slaughter as a covariate. Correlation analysis (SAS, 1990) was used to determine the associations between sow body weight loss over lactation and embryonic survival rate, between feed intake over the last week of lactation and ovulation rate, and between litter weight gain and sow body weight and backfat loss during lactation. The stepwise linear

regression procedure (SAS, 1990) was used to describe the association between progesterone concentrations at 36, 48, 72, and 96 h after onset of standing heat and ovulation rate and embryonic survival rate. For the analysis of progesterone concentrations, the SAS GLM procedure was used again (SAS, 1990), with treatment, time, and treatment × time interaction in the model. In the presence of a significant treatment × time interaction, the comparison of progesterone concentrations at 36, 48, 72, and 96 h after onset of standing heat between the two groups, and among different time points within groups was made by Duncan's multiple range test (SAS, 1990). In the results, the LSMeans and the standard errors of means (SEM) are given.

RESULTS

Of the 34 sows that were allocated to treatment during lactation, two animals became clinically ill after weaning, one did not return to estrus within 10 days after weaning, and two had poor artificial insemination success based on the breeding management records. These five sows (3 from control group and 2 from progesterone group) were removed from the experiment, resulting in data from 29 sows for final analysis. Of these, all 14 sows from the control group and 13 sows in the progesterone group were pregnant at d 28 of gestation. Two progesterone-treated sows were recorded in estrus 21 days after AI and had re-ovulated when slaughtered between d 22 and 23 after previous estrus. Therefore, in terms of reproductive characteristics, two analyses were carried out, including or excluding data from these two nonpregnant sows.

Daily feed intake during the first 3 weeks, and the last week of lactation, and during the period from weaning to d 28 of pregnancy, sow body weight, backfat thickness at P_2 at farrowing, d 21 and d 28 of lactation, changes in sow body weight, body weight loss and changes in backfat thickness from farrowing to weaning, litter weight at birth, d 21 and d 28 of age, and pig growth rate from d 1 to d 28 of lactation for the two groups were not different ($P>0.05$, Table 3.2). There was no relationship between sow body weight loss over lactation from d 1 to d 21 or from d 1 to d 28 and total or viable embryonic survival rate. Litter weight gains from birth to d 21 of lactation was correlated with sow body weight loss ($r = -0.52$, $P = 0.005$, $n = 29$), but not backfat loss during lactation ($P>0.05$).

There was no difference in progesterone concentration 36 h after onset of standing estrus ($P>0.05$), but immediately after progesterone treatment, the plasma progesterone concentrations in progesterone-treated sows increased ($P<0.001$) compared to controls and reached 19.2 ± 1.8 ng/ml 96 h after onset of standing estrus. In contrast, plasma progesterone concentrations increased gradually in controls to 6.7 ± .6 ng/ml at 96 h after the onset of standing estrus (Table 3.3). Plasma progesterone concentrations in the control group at 36 ($r = 0.52$, $P = 0.056$), 48 ($r = 0.57$, $P = 0.025$), 72 ($r = 0.71$, $P = 0.008$) and 96 h ($r = 0.65$, $P = 0.001$) after first observed standing heat were correlated with the number of CLs (Figure 3.1).

There was no difference in weaning-to-estrus interval, ovulation rate, pregnancy stage when the animals were slaughtered, and the embryo crown-rump length, between the two groups ($P>0.05$, Table 3.4). Ovulation rate was correlated with feed intake during the last week of lactation ($r = 0.38$, $n = 27$, $P = 0.053$). All measures of embryonic survival in the progesterone-treated sows were lower than in controls, but as shown in Figure 3.2, this effect was largely associated with a viable embryonic survival rate lower than 40% in seven out of fifteen progesterone-treated sows; embryo survival rates in the remaining eight sows fell within the range for control sows. Exclusion of data from the two nonpregnant sows did not affect the outcome of these analyses.

DISCUSSION

In a study in our laboratory (Zak et al., 1997), different patterns of lactational catabolism in primiparous sows produced differential effects on ovulation rate and embryonic survival. Sows on ad libitum feed intake for 21 days and 50% feed restriction during the last week of a 28-day lactation (AR) had a lower ovulation rate and embryonic survival compared to full fed sows (AA), and similar ovulation rate but lower embryonic survival than sows feed restricted for 21 days and on ad libitum feed intake during the last week of lactation (RA). The AR feeding regimen therefore selectively reduced embryonic survival. Several studies in the gilt have suggested that differences in embryonic survival rate may be mediated by differences in the concentrations of plasma progesterone in early pregnancy (Aherne and Kirkwood, 1985; Ashworth, 1991; Pharazyn et al., 1991; Jindal et al., 1996, 1997), and similar associations between plasma

progesterone concentrations in early stage of pregnancy and embryonic survival in primiparous lactating and weaned AR-treated sows were reported by Jindal (1996) and Zak et al. (1998). Therefore, this same sow model was used to directly examine the role of progesterone as a mediator of embryonic survival rate in the primiparous weaned sow.

In the present experiment, the sows lost more backfat (5.6-5.7 mm vs 4.7 mm) and body weight during lactation compared to the AR sows in previous studies (Jindal, 1996; Zak et al., 1997). Litter size is a major factor determining milk production and hence the nutrient requirements of the sow. The influence of litter size (6 vs 10) on backfat and body weight loss in a 28-day lactation in primiparous sows has been shown by Yang et al. (1989). The number of pigs suckling in the present study was increased from 6 to 9 per litter, so that the AR treatment would more closely reflect the commercial situation. This increase in pigs suckling resulted in a 54% increase in total litter weight gain compared to AR sows suckling only 6 pigs (Zak et al., 1997). In turn, the extra milk production associated with this change resulted in greater mobilization of maternal tissue, as evidenced by an extra 2.9% in sow body weight loss and an extra 5.3% increase in backfat loss over the 28-d lactation, and an extra increase in negative energy balance by 763 Kcal of ME/d (-4457 vs -3694 Kcal of ME/d), based on the energy calculation method of lactating sows recommended by Noblet et al. (1989). A reduction in feed intake during the last week of lactation was also reported to increase the fat content of milk, and the fatty acid pattern of milk may change due to excessive mobilization of body lipid and protein (Mullan and Williams, 1989). Though milk fat was not estimated, the litter weight gain from birth to day 21 of lactation had a strong negative correlation with sow body weight loss ($r = -0.52$, $P = 0.005$) but not backfat during lactation.

In the present study, the average weaning-to-estrus intervals of 123.5 to 125.0 h were similar to intervals of 122.4 and 127.2 h observed previously (Jindal, 1996; Zak et al., 1997). Consistent with the study of Yang et al. (1989), the increase in litter size from six to nine did not seem to influence the interval from weaning to estrus in primiparous sows. The average ovulation rates of 18.1 and 18.3 were higher than those of 15.4 observed by Jindal (1996) and 15.6 reported by Zak et al. (1997) using the same genetic population. This increase in ovulation rate may be related to higher feed intakes allowed during the last week of lactation in the current study (3.3 and 3.1 kg) than in previous

studies (2.7 kg, Jindal, 1996; 2.1 kg, Zak et al., 1997), as a consequence of high voluntary feed intake between d 17 and 21 of lactation. As suggested by Brooks (1982), sows that become catabolic during lactation may remain so after weaning and as a consequence have reduced ovulation rates. Indeed, there was a reasonable correlation between the feed intake during the last week of lactation and ovulation rate ($r = 0.38$, $P = 0.053$) in the present experiment.

Consistent with data from Webel et al. (1975) obtained at d 9 of pregnancy, plasma progesterone concentrations at 48, 72 and 96 h after onset of standing heat in control animals were correlated with the number of CLs (Figure 3.1). Although there was no correlation between plasma progesterone concentration at 36, 48, 72, or 96 h after onset of standing estrus in control sows with embryonic survival rate, likely because of the limited number of animals used, the distribution of data shown in Figure 3.1 is similar to that reported in AR sows by Jindal (1996). The viable embryonic survival rate in controls was slightly higher than, but comparable to, that observed previously in AR treatment groups (58.5%, Jindal, 1996; 64.4%, Zak et al., 1997). The total embryonic survival rate and the viable embryo survival rate in progesterone sows were lower than in controls ($P<0.01$). These results were obviously not consistent with the positive responses to progesterone therapy reported in gilts (Ashworth, 1991; Jindal et al., 1997).

Compared with the gilt model, in which progesterone treatment started from 24 h after standing estrus, i.e. 15 h after the LH peak or 21 h before ovulation (Jindal et al., 1997), in the present study based on data from the same sow genotype in the study of Clowes (1993), the sows were treated with progesterone from 36 h after standing estrus, i.e. 18 h after the LH peak or 18 h before ovulation. Thus, in relation to the time of the LH peak and ovulation, the progesterone treatment procedure was approximately the same as in gilts. Furthermore, the exogenous progesterone treatment increased peripheral progesterone concentrations to physiologically high range for the early luteal phase. Although calculation of embryo survival is based on the assumption that fertilization rate is 100%, some component of this estimate of embryo mortality is related to fertilization failure (Lambert et al., 1991; Kemp and Soede, 1997). Day and Polge (1968) reported that the percentage of eggs fertilized was reduced, and the incidence of polyspermy was greatly increased, in gilt injected with progesterone 24 or 36 h before ovulation, and it is

therefore possible that progesterone may also have adversely affected fertilization rate. Assuming that the duration of estrus in weaned sows with an early return to estrus would be at least 3 days, and therefore, probably a day longer than we observe in the cyclic gilt, the amount of variation in the interval between onset of standing estrus and ovulation may be more variable. Therefore, the time of treatment in relation to the time of ovulation might fall into the 24-36 h window described in the study of Day and Polge (1968). In this case, it is possible that fertilization failure may have been an important factor in sows returning to estrus after breeding, or with low embryo numbers at d 28.

Embryos are normally retained within the oviduct for about two days and secretions in the oviduct may affect cleavage rate or embryonic viability (Fukui et al., 1988; Gandolfi and Moor, 1987). In addition, the time spent in the oviduct may also be necessary to give the uterus time to prepare to nurture the embryo. Eventually rising progesterone concentrations seem to cause dilation of the oviduct and as a result, transport of the embryos to the uterus (Dziuk, 1985). Generally, estradiol retards, whereas progesterone enhances, the transport of the fertilized eggs. Supplementation with exogenous progesterone during this time can reduce the time that embryos reside in the oviduct (Day and Polge, 1968) and in turn affect embryonic development. Secretory activity of the endometrium is also correlated with the endocrine status of the dam. Protein synthesis by the endometrium is high when the ratio of progesterone to estrogen is at a maximum and progesterone treatment during early gestation caused a significant increase in the amount of uterine proteins (Knight et al., 1974; Roberts and Bazer, 1988). Some of the proteins play a nutritional role, and some also play a part in directing or limiting the growth and development of the conceptus (Roberts and Bazer, 1988). The study by Lawson and Cahill (1983) demonstrated that high levels of progesterone during the first 4 days of the estrous cycle of the ewe hastened the development of the dioestrus uterus so that on day 6 it was able to provide an environment acceptable to 10-day old embryos. In pigs, it is well established that embryos transferred to more advanced uteri quickly die (Jarrell et al., 1990; Geisert et al., 1991). Thus progesterone may have led to an asynchrony between embryonic development and uterine function and some embryonic losses may simply result from the inability of less-developed embryos to maintain their rates of development in the face of an increasingly hostile uterine

environment. Again, the very divergent effects of progesterone treatment in the sows in this experiment, compared to the effects in gilts reported previously, may relate to differences in the time relative to ovulation at which treatment was given. With high quality, transrectal ultrasonography now available in our laboratory, it will be possible in future experiments to address this question.

In conclusion, compared with previous experiments in gilts, a similar pattern of progesterone therapy actually decreased embryonic survival rate in this study, rather than reversing the detrimental effect of increased catabolism in late lactation on embryonic survival.

LITERATURE CITED

Aherne FX, Kirkwood RN. Nutrition and sow prolificacy. J Reprod Fertil Suppl 1985; 33: 169.

Ashworth CJ. Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. Anim Reprod Sci 1991; 26: 311-321.

Ashworth CJ, Sales DI, Wilmut IW. Evidence of an association between the survival of embryos and the periovulatory plasma progesterone concentration in the ewe. J Reprod Fertil 1989; 87: 23.

Brooks PH. The gilt for breeding and for meat. In: Cole DLA, Foxcroft GR (eds.), Control of Pig Reproduction. Butterworths, London. 1982; 211-224.

Clowes EJ. Metabolic indicators in post-weaning sow fertility. Edmonton, CA: University of Alberta, 1993. M.Sc. Thesis.

Day BN, Polge C. Effects of progesterone on fertilization and egg transport in the pig. J Reprod Fertil 1968; 17: 227-230.

Den Hartog LA, van der Steen HAM. Reproductive traits in primiparous sows. Neth J Agric Sci 1981; 29: 285.

Dyck GW, Strain JH. Postmating feeding level effects on conception rate and embryonic survival in gilts. Can J Anim Sci 1983; 63: 579.

Dziuk PJ. Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival. J Reprod Fertil 1985; 33: 57-63.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Fukui Y, Glew AM, Gandolfi F, Moor RM. *In vitro* culture of sheep oocytes matured and fertilized *in vitro*. Theriogenology 1988; 29: 883-891.

Gandolfi F, Moor RM. Stimulation of early embryonic development in the sheep by co-culture with oviduct epithelial cells. J Reprod Fertil 1987; 81:23-28.

Geisert RD, Morgan GL, Zavy MT, Blair RM, Gries LK, Cox A, Yellin T. Effect of asynchronous transfer and estrogen administration and development of porcine embryos. J Reprod Fertil 1991; 93: 475-481.

Jarrell VL, Beckmann LS, Cantley TC, Rieke AR, Day BN. The effect of exposure to an asynchronous uterus on development of day 4 swine embryos. J Anim Sci 1990; 68 (Suppl.1): 429.

Jindal R. Physiological mechanisms mediating nutritional effects on embryonic survival in the pig. Edmonton, CA: University of Alberta: 1996. Ph.D. Thesis.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR. Effect of nutrition on embryonic mortality in gilts: Association with progesterone. J Anim Sci 1996; 74: 620-624.

Jindal R, Cosgrove JR, Foxcroft GR. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. J Anim Sci 1997; 75: 1063-1070.

Kemp B, Soede NM. Consequences of variation in interval from insemination to ovulation on fertilization in pigs. J Reprod Fertil Suppl 1997; 52: 79-89.

Kirkwood RN, Thacker PA. Nutritional factors affecting embryonic mortality in pigs (results and speculation). Pig News and Inf 1988; 9: 15-21.

Knight JW, Bazer FW, Wallace HD. Effect of progesterone induced increase in uterine secretory activity on development of porcine conceptus. J Anim Sci 1974; 39: 743.

Lambert E, Williams DH, Lynch PB, Hanrahan TJ, McGeady TA, Austin FH, Bolarnd MP, Roche JF. The extent and time of prenatal loss in gilts. Theriogenology 1991; 36: 655-665.

Lawson RA, Cahill LP. Modification of the embryo-maternal relationship in ewes by progesterone treatment early in the oestrous cycle. J Reprod Fertil 1983; 67: 473-475.

Mullan BP, Williams IH. The effect of body reserves at farrowing on the reproductive performance of first-litter sows. Anim Prod 1989; 48: 449-457.

Noblet J, Dourmad JY, Etienne M. Energy utilization in pregnant and lactating sows: modeling of energy requirements. J Anim Sci 1989; 68: 562-572.

NRC. Nutrient Requirements of Swine (9th ed.). National Academy Press, Washington, DC. 1998.

Parr RA, Davis IF, Fairclough RJ, Miles MA. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. J Reprod Fertil 1987; 80:317-320.

Parr RA, Hughes PE, Davis IF, Miles MA, Ferlazza JC. Nutrition, progesterone and the early pig embryo. Proc 4th Int Conf Pig Reprod, Columbia. 1993; 93 (Abstract).

Pharazyn, A., den Hartog LA, Foxcroft GR, Aherne FX. Dietary energy and protein intake, plasma progesterone and embryo survival in early pregnancy in the gilt. Can J Anim Sci 1991; 71: 949.

Roberts RM, Bazer FW. The functions of uterine secretions. J Reprod Fertil 1988; 82: 875-892.

SAS. User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC. 1990

van den Brand H, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: II. Effects on periestrus hormone profiles and embryonal survival. J Anim Sci 2000; 78: 405-411.

Webel SK, Reimer TJ, Dziuk PJ. The lack of relationship between plasma progesterone levels and number of embryos and their survival in the pig. Biol Reprod 1975; 13: 177-186.

Yang H, Eastham PR, Phillips P, Whittemore CT. Reproductive performance, body weight and body condition of breeding sows with differing body fatness at parturition, differing nutrition during lactation, and differing litter size. Anim Prod 1989; 48: 181-201.

Zak LJ, Cosgrove JR, Aherne FX, Foxcroft GR. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J Anim Sci 1997; 75: 208-216.

Zak LJ, Williams IH, Foxcroft GR, Pluske JR, Cegielski AC, Clowes EJ, Aherne FX. Feeding lactating primiparous sows to establish three divergent metabolic states: I. Associated endocrine changes and postweaning reproductive performance. J Anim Sci 1998; 76: 1145-1153.

Table 3.1. Composition of experimental diets as formulated

Item	Lactating sow	Gestating sow
Ingredient, %		
Barley	79.4	84.5
Soybean meal	12.5	5
Lysine	0.2	--
Canola	--	5
Limestone	1.4	1.4
Tallow	3	1
Vitamin-mineral premix*	1	1
Iodized salt	0.5	0.5
Chemical analysis		
Digestible energy, Kcal/kg	3410	3045
Crude protein %	16.05	13.89
Lysine %	0.87	0.6
Ca %	1	0.92
P %	0.83	0.71

*: Supplied the following per kg of diet: Zn, 120 mg; Mn, 12 mg; Fe, 150 mg; Cu, 12 mg; Se, .1 mg; vitamin A, 5,000 IU; vitamin D_3, 500 IU; vitamin E, 22 IU; riboflavin, 12 mg; niacin, 45 mg; calcium pantothenate, 24 mg; choline chloride, 840 mg; vitamin B_{12}, 30 ug; biotin, 200 ug.

Table 3.2. LSMeans (± SEM) of sow feed intake during and after lactation, sow body weight and body weight change, sow backfat thickness at P_2 and backfat thickness change, litter weight and piglet growth rate during lactation in control and progesterone-treated sows

	TREATMENT	
Item	Control (n = 14)	Progesterone (n = 15)
Sow feed intake (kg/d)		
D 1 to 21	5.8 ± 0.2	5.5 ± 0.2
D 22 to 28	3.3 ± 0.1	3.2 ± 0.1
Weaning to slaughtering	5.7 ± 0.2	5.9 ± 0.2
Sow body weight (kg)		
Farrowing	201.0 ± 2.7	199.4 ± 2.8
D 21	191.6 ± 3.2	189.1 ± 3.3
D 28	173.6 ± 3.3	171.1 ± 3.4
Sow weight change, kg, d 1 to 28	-27.4 ± 2.3	-28.3 ± 2.3
Sow body loss (%)	13.6 ± 1.2	14.1 ± 1.1
Sow backfat (mm)		
Farrowing	18.4 ± 0.8	18.6 ± 0.8
D 21	15.4 ± 0.7	15.7 ± 0.8
D 28	12.8 ± 0.7	12.9 ± 0.7
Sow backfat change, d 1 to 28	-5.6 ± 0.5	-5.7 ± 0.5
Piglets weaned	8.9 ± 0.1	8.7 ± 0.1
Litter weight (kg)		
D 1	16.6 ± 0.7	15.9 ± 0.7
D 21	67.1 ± 2.0	65.0 ± 2.0
D 28	82.8 ± 2.1	80.5 ± 2.1
Growth rate (g/d, d1 to 28)	271.6 ± 7.4	274.9 ± 7.4

Table 3.3. LSMeans (± SEM) of plasma progesterone concentrations (ng/ml) at 36, 48, 72, and 96 h after onset of standing heat in the control (n = 14) and progesterone-treated sows (n = 15)

Treatment	36 h	48 h	72 h	96 h
Control	$0.7 \pm 0.2^{a,x}$	$1.5 \pm 0.3^{a,x}$	$3.9 \pm 0.5^{a,y}$	$6.7 \pm 0.6^{a,z}$
Progesterone	$0.6 \pm 0.1^{a,w}$	$6.3 \pm 0.5^{b,x}$	$12.4 \pm 0.7^{b,y}$	$19.2 \pm 1.8^{b,z}$

^{a,b}: Within a column, means lacking a common superscript differ ($P<0.001$);

^{w,x,y,z}: Within a row, means lacking a common superscript differ ($P< 0.03$).

Table 3.4. LSMeans (± SEM) of weaning-to-estrus interval (WEI), ovulation rate, total number of embryos, total embryonic survival rate, number of viable embryos, viable embryonic survival rate and crown-rump length at day 28 ± 3 of pregnancy in control and progesterone-treated sows

	Control group	Progesterone-treated group	
Item	All pregnant sows (n = 14)	All sows (n = 15)	Pregnant sows (n = 13)
WEI (h)	123.5 ± 5.7	123.1 ± 5.9	125.0 ± 7.4
Pregnant stage (day)	26.6 ± 0.2	NA[c]	26.8 ± 0.3
Ovulation rate (CLs)	18.1 ± 0.4	NA	18.3 ± 0.5
Total number of embryos	13.3 ± 1.0[a]	8.5 ± 1.1[b]	9.8 ± 1.2[b]
Total embryonic survival (%)	73.1 ± 4.7[a]	47.3 ± 5.4[b]	54.4 ± 5.8[b]
Number of viable embryos	12.6 ± 0.9[a]	7.1 ± 0.9[b]	8.2 ± 1.0[b]
Viable embryonic survival (%)	69.5 ± 4.2[a]	39.7 ± 4.8[b]	45.2 ± 5.2[b]
Crown-rump length (mm)	21.0 ± 0.7	NA	21.4 ± 0.7

[a, b]: Means within a row with different superscripts differ ($P < 0.005$);

[c]NA = data not available because of two non-pregnant sows.

Figure 3.1. Correlation between the number of corpora lutea and progesterone concentrations at 72 h after onset of standing heat in the control sows (n = 14).

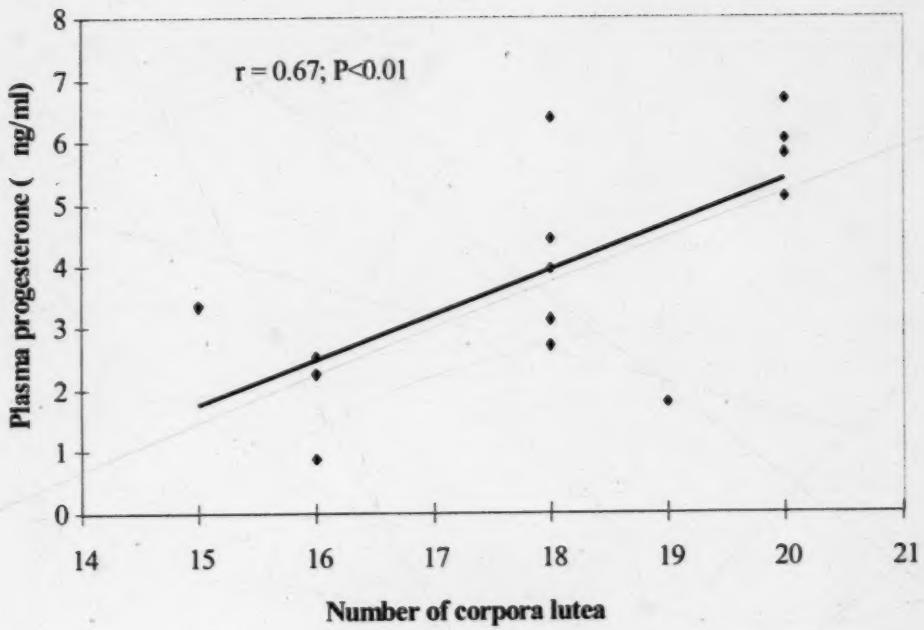
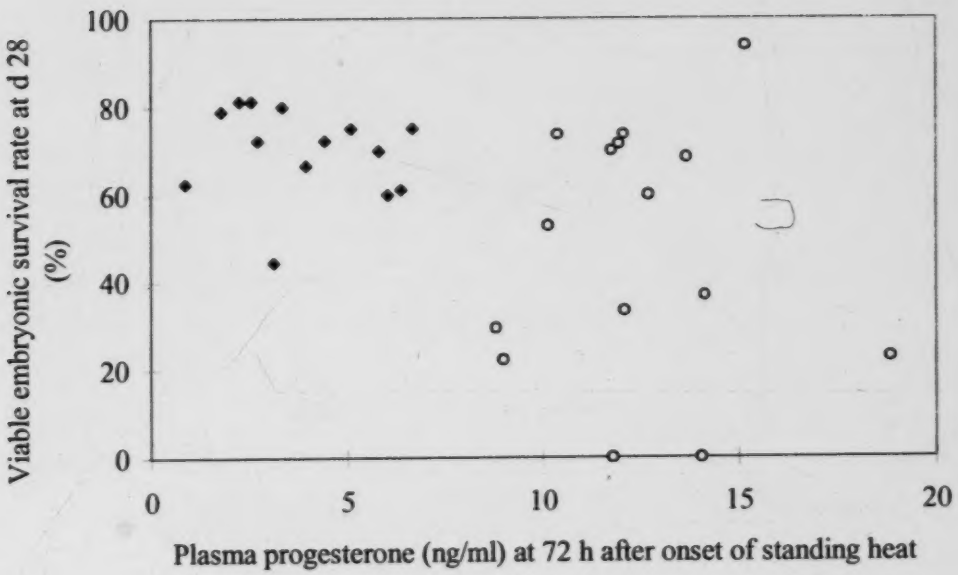

r = 0.67; P<0.01
Plasma progesterone (ng/ml)
0
1
2
3
4
5
6
7
8
14
15
16
17
18
19
20
21
Number of corpora lutea

Figure 3.2. Relationship between plasma progesterone at 72 h after onset of standing heat and embryonic survival rate in all sows (n = 29) (Control sows: ♦; Progesterone-treated sows: o).


Viable embryonic survival rate at d 28 (%)
100
80
60
40
20
0
0
5
10
15
20
Plasma progesterone (ng/ml) at 72 h after onset of standing heat

CHAPTER 4

REPRODUCTIVE, METABOLIC, AND ENDOCRINE RESPONSES TO FEED RESTRICTION AND GnRH TREATMENT IN PRIMIPAROUS LACTATING SOWS

INTRODUCTION

The relationship between nutrition and reproduction in swine has been extensively reviewed (Foxcroft et al., 1995; Quesnel and Prunier, 1995; Foxcroft, 1997). Nutrition may influence reproductive performance by a number of mechanisms including central effects on gonadotropin secretion (Booth et al., 1994) and local effects on ovarian function (Cosgrove et al., 1992; Cosgrove and Foxcroft, 1996). Zak et al. (1997a) and Quesnel et al. (1998) have reported an inhibitory effect of short-term, severe feed restriction on pulsatile LH secretion in lactating sows. The data of Zak et al. (1997a) showed that when primiparous sows were fed to appetite from farrowing to d 21 of lactation, but then restricted to 50% of ad libitum intake from d 22 to 28, embryonic survival at d 28 of gestation decreased to 64.4%, compared to 87.5% in sows fed to appetite throughout the lactation, and to 86.5% in sows feed restricted from farrowing to d 21 and fed to appetite from d 22 to weaning; both periods of feed restriction reduced ovulation rate compared to sows fed to appetite. Feed restriction during the last week of lactation, therefore, selectively reduced embryo survival rate, independent of ovulation rate. Feed restriction during lactation has also been shown to affect follicular development at weaning and at 48 h after weaning (Quesnel et al., 1998) and affect developmental competence of oocytes collected 4 days after weaning and cultured *in vitro* (Zak et al., 1997b). Reduced embryo survival in sows, as a result of reduced feed intake during lactation may, therefore, be primarily related to reduced gonadotropin secretion and inadequate follicular maturation (Foxcroft et al., 1995; Zak et al., 1997a; Quesnel et al., 1998).

Taking advantage of the experimental paradigm developed by Zak et al. (1997a), the present study determined whether use of exogenous GnRH treatment in feed-

---

† : A paper based on Chapter 4 has been published in the Journal of Animal Science (1999, 77:724-735) by Mao J, Zak LJ, Cosgrove JR, Shostak S, and Foxcroft GR.

restricted sows, which restored LH secretion to that seen in unrestricted sows in late lactation, would ameliorate effects of restrict feeding on subsequent reproductive performance.

MATERIALS AND METHODS

Animal and Treatments

Forty-one primiparous Camborough sows (Pig Improvement [Canada] Ltd.), were allocated as equally as possible with respect to sow body weight at farrowing and weight loss over the first three weeks of lactation, to one of three treatments. All sows were kept in barns with a totally controlled environment and fed three times daily at 0800, 1130, and 1500 throughout a 28-d lactation with a barley-soybean diet formulated to provide 3,200 kcal of ME/kg, 15.86% crude protein, and .86% lysine (Table 4.1). Within 48 h after farrowing, the number of pigs was standardized to 8 to 10 per litter by cross fostering. Creep feed was not available. AA sows were fed to appetite throughout a 28-d lactation (Group AA). AR sows were fed to appetite from farrowing until d 21 and then restricted to 50% of the average consumption of the sows fed to appetite from d 22 to 28 (Group AR). Based on the data of De Rensis et al. (1991), AR + GnRH sows were fed as AR sows and received 800 ng of GnRH (Catalog #: L7134, Sigma Chemical Co. St. Louis, MO) in 4 ml saline i.v. at 6-h intervals from d 22 to 28 (Group AR + GnRH), while AA and AR sows received saline alone. All feed consumption was determined on a per-meal basis. Water was available ad libitum to both the sows and pigs throughout the experimental period. Sow body weight, backfat thickness at P_2 (Scanoprobe II, Scano, Ithaca, NY), and litter weight were recorded at weekly intervals throughout lactation.

From weaning until mating all sows were fed ad libitum with a barley-soybean diet formulated to provide 3,000 kcal of ME/kg, 13.75% crude protein, and .56% lysine (Table 4.1). After mating, all sows were fed the same diet to meet NRC (1988) recommended nutritional requirements. Onset of standing estrus was detected twice daily at 0730 and 1930 by direct exposure to mature vasectomized boars for 15 min to determine weaning-to-estrus interval; mating was not permitted. The 24-h period after first detection of estrus was designated as d 0 of gestation. All experimental sows were artificially inseminated 12 and 24 h after the first observed standing estrus with pooled

semen (3×10^9 spermatozoa/dose) from the same group of three fertile boars specifically designated for this experiment (Alberta Swine Genetics Cooperation, Leduc, AB, Canada). The same two trained persons inseminated all sows. The research protocol was approved in accordance with the Canadian Council on Animal Care guidelines.

Ovulation Rate and Embryonic Survival

All sows were slaughtered at a local abattoir on d 28 ± 3 of pregnancy. Immediately after slaughter, the reproductive tract was recovered from each sow and both ovaries were examined to determined the number of corpora lutea (ovulation rate). Several cuts into the ovarian tissue were made to determine the presence of any hidden corpora lutea. Each uterine horn was opened by blunt dissection along the antimesometrial axis and gentle eversion of the uterus was used to recover embryos within their trophoblastic vesicles. To provide an objective measure of abnormal development, embryos were classified as being nonviable on the basis of a crown-rump length significantly less than the mean for all embryos recovered from that sow (Jindal et al., 1996). Embryonic survival was expressed as the percentage of corpora lutea represented by normally developed embryos.

Blood Sampling

At d 17 of lactation an indwelling jugular catheter was implanted surgically, under general anesthesia, via the superficial cephalic vein (Cosgrove et al., 1993). Three ml blood samples were withdrawn at 15-min intervals from 0800 to 2000 on d 21, and for two 12-h periods before and after weaning at 2000 on d 28, for analysis of LH. Additional 5 ml samples were collected hourly for analysis of IGF-I, insulin, and FSH by RIA. In addition two samples were taken before morning feeding (at 0600 and 0700) for preprandial insulin and leptin assay. Blood samples were collected into heparinized tubes, centrifuged at $1,500 \times g$ for 15 min, and the plasma decanted and stored at -30 °C until analysis. To determine the effect of feeding to appetite and feed restriction for 7 d on plasma leptin concentrations, the two preprandial samples collected at 0600 and 0700, two postprandial samples at 1600 and 1700 on d 21 and 28, and two morning samples at 0600 and 0700 on the day after weaning were used for leptin assay. Indwelling catheters were withdrawn at the end of the intensive sampling period.

Estimation of Plasma LH, FSH, Insulin, IGF-I, and Leptin

For RIA analysis, all treatment groups were represented in each assay, and all samples from a sow were analyzed in the same assay. Assay sensitivity was calculated as the concentration at two standard deviations below the percentage of total binding. Plasma LH and FSH concentrations were determined in duplicate using the homologous double antibody RIAs described previously by Cosgrove et al. (1991). For LH, 200 μL of plasma was assayed, the intra- and inter-assay CV were 6.0 and 14.2 %, respectively, and average sensitivity was 94.4% of total binding and equal to 0.006 ng/tube. The LH potencies are expressed as nanogram equivalents of SDG-2-65 pLH (.96-1.18 × NIH oLH S19). For FSH, 300 μL of plasma was assayed, the intra-assay CV for the single assay used was 8.5%, and sensitivity was 94.2% of total binding and equal to 0.78 ng/tube. The FSH potencies are expressed as nanogram equivalents of USDA-pFSH-B-1 (AFP-5600; 2.0 × S1 U/mg).

Plasma insulin and IGF-I concentrations were determined in duplicate using the homologous double antibody RIAs described previously by Cosgrove et al. (1992). For insulin, 100 μL of plasma was assayed, the intra-assay CV for the single assay was 4.2%, and sensitivity was 96.0% of total binding and equal to 0.013 ng/tube. For IGF-I, 100 μL of sample was extracted, the cold recovery efficiency and intra-assay CV were 102.6% and 4.0%, respectively, and sensitivity was 97.1% of total binding and equal to 0.003 ng/tube. Plasma leptin concentrations were measured in duplicate using the multi-species double antibody kit assay (Linco Research, St. Louis, MO; Catalog #: XL-85K) with some modifications. Modifications included the doubling of the original sample and assay buffer volume because of low levels of leptin in these samples and use of 0.1% egg white buffer (0.1% egg white in PBS/Tween 20/EDTA, pH 7.4) for the buffer step and for diluting control plasma. The intra-assay CV for the single assay used was 4.2%, and sensitivity was 94.5% of total binding and equal to 0.22 ng/tube.

Blood Sampling for Progesterone Assessment

On the basis of earlier data, blood samples (2.5 ml) were collected from a peripheral ear vein of each sow into heparinized tubes at 36, 48, 72, and 96 h after first detection of standing estrus during a brief period of nose-snare restraint (Jindal et al.,

1996). Plasma was harvested by centrifugation at 1,500 × g for 15 min immediately after collection and stored at -30°C until analysis for progesterone concentration using a Coat-A-Count progesterone kit (Diagnostic Products Corporation, Los Angeles, CA; Catalog #: TKPG1) validated for use as a direct assay with porcine plasma (Chapter 3). The sensitivity of the assay was 96.5% of total binding and equal to 0.01 ng/tube (0.1 ng/ml).

Statistical Analyses

All dependent variables were analyzed for normality using the Wilk-Shapiro test (SAS, 1990). Data for the dependent variables, sow body weight, backfat thickness, litter weight, FSH, insulin, IGF-I, leptin across day, and progesterone at 36, 48, 72, and 96 h after onset of standing heat were analyzed by the repeated measures GLM procedure of SAS (1990). For all dependent variables, sources of variation were block (farrowing group), treatment, sow within block by treatment interaction, and the repeated measures of day. Analysis for sow body weight, backfat, and litter weight at d 7, 14, 21, and 28 also included, as a covariate, sow weight, backfat, and litter weight at farrowing, respectively. Significant differences among treatments were determined using sow within block by treatment interaction as the error term. In the event of a significant day by treatment interaction, differences among days were determined within treatments and differences among treatments determined within days. Weaning-to-estrus interval, ovulation rate, embryonic survival rate, sow body weight change over lactation, backfat thickness change, and pig growth rate were analyzed using analysis of variance, fitting treatment, and block as the main effects. Sow was the experimental unit and used as the error term. Differences among treatment means were determined using Duncan's multiple range test (SAS, 1990). Comparisons of plasma insulin concentrations among treatment groups on d 21 and 28 were made using means from two time periods: preprandial (2 h before the morning feed after an overnight fast) and prandial/postprandial (the mean of 12 samples collected from 0800 to 1900 with feeding at 0800, 1130, and 1500). After weaning, the mean of all 12 hourly samples were used in the analysis (Zak et al., 1997a). The average crown-rump length of all embryos from each sow was adjusted to d 28 using day of gestation at slaughter as a covariate. Correlation analysis (SAS, 1990) was used to determine the associations between plasma maximum,

mean and minimum LH concentrations and weaning-to-estrus interval, and associations between plasma insulin and leptin concentrations, plasma leptin and mean LH concentrations on d 21 and 28. The association analysis between progesterone concentrations at 36, 48, 72, and 96 h after onset of standing estrus, and ovulation rate and embryonic survival rate, was performed using stepwise linear regression analysis (SAS, 1990). In the results, the LSMeans and the standard errors of LSMeans are given.

Plasma LH data were characteristized initially by the sliding window technique of Shaw and Foxcroft (1985), and maximum, mean, and minimum LH concentrations at d 21, and at d 28 before and after weaning, were used for statistical analysis. When appropriate, LH episodic frequency was appraised visually according to the criteria established by Cosgrove et al. (1991). An LH episode was defined as any increase in LH concentration within 2 sampling intervals, followed by a decline, with at least 3 sampling intervals between the defined peak and succeeding baseline, occurring at a rate approximating the half-life of the hormone.

RESULTS

Forty-one primiparous sows composed of four farrowing groups (blocks) were allocated initially to treatment. Four sows (one from AA and three from AR + GnRH group) were clinically ill during the experimental period and withdrawn from the study. Four sows (two each from the AA and AR group, respectively) did not return to estrus within 10 d after weaning. Ovarian characteristics of these sows showed that two (one each from the AA and AR group) had ovulated before weaning, and two had no medium or large sized follicles on their ovaries when they were killed on d 12 after weaning. One sow from the AA group recycled 20 d after breeding and its uterus contained a term-sized, degenerating fetus that was obviously retained in utero after farrowing. Excluding these five animals, production and reproductive performance data from 32 sows were used for analysis. One sow from the AR + GnRH group was pregnant, but slaughtered on d 15 of gestation for health reasons, and ovulation rate and embryo survival data from this sow were also excluded from the analysis (n = 31). Catheters were lost in three sows and LH, FSH, insulin, IGF-I, and leptin data from 29 sows were used for the analysis of endocrine characteristics.

Sow Body Weight, Backfat, Metabolic Status, and Litter Performance

Based on the work of Noblet et al. (1990), sow metabolic status was determined during lactation according to the formula:

$$EB = FI \times ED - [22.0 \times BW + 6.83 \times LG - 125 \times n + 1{,}430]$$

where EB represents the energy balance (kcal ME/d); FI, feed intake (kg); ED, energy density in the feed (kcal ME/kg); BW, mean sow body weight over the period (kg); LG, the gain of the litter over the period (g/d); and n, the number of pigs in a litter. Sow feed intake, body weight and weight change, backfat thickness at P_2 and backfat change, metabolic status, and pig growth rate are summarized in Table 4.2.

Sow body weight and backfat thickness did not differ among treatment groups at farrowing ($P > 0.05$). There was no difference in feed consumption among the three groups over the first 3 wk of lactation. Over the last week of lactation, AA sows increased their feed consumption, compared to that of the first 3 wk ($P < 0.05$). As a result of feed restriction, feed consumption in both AR and AR + GnRH sows was lower than that in AA sows during that period ($P < 0.01$). However, during the weaning to mating period the restricted sows consumed more feed than AA sows ($P < 0.05$). All sows exhibited a loss of body weight and backfat thickness during lactation ($P < 0.01$). Although, there was no difference in body weight loss between AR and AR + GnRH sows ($P > 0.05$), these restricted sows lost more body weight than AA sows ($P < 0.02$). In contrast, there was no difference in backfat loss among the three groups. There was no difference in the number of pigs suckling ($P > 0.05$). Pig growth rate over the first 3 wk of lactation was not different among the treatments ($P > 0.05$). However, pigs suckling AA sows over the last week of lactation grew faster than those suckling AR and AR + GnRH sows ($P < 0.01$). As a result, the overall pig growth rate over lactation from AA sows was higher than that of pigs from both AR and AR + GnRH sows ($P < 0.03$). During the first 3 wk of lactation, there was no difference in energy balance among the groups ($P > 0.05$), but as a result of feed restriction, energy balance in AR and AR + GnRH sows was lower than in AA sows ($P < 0.01$).

Plasma Insulin, IGF-I, Leptin, LH, and FSH Characterization

Insulin. There was a significant day by treatment interaction ($P < 0.01$) for plasma insulin concentration. Analysis of day within treatment revealed no difference in prandial/postprandial plasma insulin concentration at both d 21 and 28 in AA sows, but prandial/postprandial insulin concentrations were lower ($P < 0.001$) at d 28 than at d 21 in both AR and AR + GnRH sows (Table 4.3). Immediately after weaning, plasma insulin concentrations decreased ($P < 0.01$) in AA sows; in AR and AR + GnRH sows weaning produced no change in insulin concentrations. Analysis of treatment within day revealed no differences in preprandial insulin concentrations at d 21 or 28 of lactation ($P > 0.05$). Plasma prandial/postprandial insulin concentrations at d 21 did not differ among the treatments, however, plasma insulin concentrations at d 28 in AR and AR + GnRH sows were lower ($P < 0.01$) than in AA sows. After weaning no differences in plasma insulin concentrations were observed among treatments.

IGF-I. There was a significant day by treatment interaction for plasma IGF-I ($P < 0.001$). Mean plasma IGF-I concentration was similar at d 21 of lactation and d 28 before and after weaning for AA sows, whereas mean plasma IGF-I concentration decreased from d 21 to 28 in group AR and AR + GnRH ($P < 0.001$) and did not change after weaning (Table 4.3). Analysis of treatment within day revealed that mean plasma IGF-I concentration at d 28 was lower in AR and AR + GnRH than in AA sows both before ($P < 0.002$) and after ($P < 0.01$) weaning.

Leptin. There was a significant day by treatment interaction for plasma leptin ($P < 0.0001$). Plasma leptin concentrations on d 21 followed the same pattern among the three treatments and no difference was observed. The data from those three groups were pooled and summarized in Figure 4.1. The postprandial leptin concentrations on d 21 after feeding to appetite three times were higher compared to the preprandial period ($P < 0.0001$). Similarly postprandial leptin concentrations on d 28 in AA sows increased compared to the levels before feeding ($P < 0.0001$), and decreased after the 15 h fast on d 28 after weaning. However, no change was observed in AR and AR + GnRH sows on d 28 after feeding ($P > 0.05$), and the postprandial leptin concentrations in AR and AR + GnRH sows were lower on d 28 after 7 d feed restriction than those in AA sows ($P < 0.001$). After 15 h fast on d 28, the leptin concentrations in AA sows decreased ($P <$

0.003) and were not different between groups. Significant associations were established between plasma insulin and leptin concentrations ($r = 0.24$, $P < 0.004$), and between plasma leptin and mean LH concentrations on both d 21 and 28 ($r = 0.47$, $P < 0.0003$).

LH. There was a day by treatment interaction for maximum, mean, and minimum LH concentrations and LH pulse frequency ($P < 0.05$). Analysis of treatment within day showed that there was no difference in plasma LH maximum, mean, and minimum concentrations or LH pulse frequency at d 21 among the treatments ($P > 0.05$) (Figure 4.2, Table 4.3). On d 28, compared to AA sows, feed restriction in AR sows did not influence LH mean and minimum concentrations, but decreased LH maximum and LH pulse frequency ($P < 0.05$). However, there was no difference in LH characteristics between AA and AR + GnRH sows ($P > 0.05$). After weaning, there was no difference in LH secretion between AA and AR, or between AR and AR + GnRH sows, but there was a difference in all the characteristics of LH secretion between AA and AR + GnRH sows ($P < 0.05$). Both AA and AR sows showed an increase ($P < 0.04$) in mean and minimum LH concentrations, and likely in LH pulse frequency (Figure 4.3), in response to weaning, although the pattern of LH secretion after weaning in both groups did not allow objective estimation of LH pulse frequency. However, no LH changes in response to weaning were observed in AR + GnRH sows.

FSH. There was no treatment by day interaction for plasma FSH concentration, but there was a day effect on FSH concentrations ($P < 0.03$). The analysis of day within treatment revealed that there was no change in plasma FSH concentration among d 21, d 28, and post-weaning period in AA sows ($P > 0.05$) (Figure 4.2). Feed restriction tended to decrease plasma FSH concentrations in both AR and AR + GnRH sows at d 28 compared to d 21 of lactation ($P < 0.06$). After weaning, there was no change in FSH concentrations in AA and AR + GnRH sows, but plasma FSH in AR sows increased from 33.9 ± 3.0 before, to 40.8 ± 2.1 ng/ml after, weaning ($P < 0.05$).

Reproductive Performance

Reproductive characteristics are summarized in Table 4.4. Weaning-to-estrus interval in AA sows was shorter than that in AR and AR + GnRH sows ($P < 0.05$), but was not different between AR and AR + GnRH sows ($P > 0.05$). Ovulation rate, embryonic survival rate, and embryo size did not differ among the three treatment groups

($P > 0.05$). Plasma progesterone concentrations at 36, 48, and 72 h after onset of standing heat were not different among treatments (Table 4.5). However, progesterone concentrations in AR sows at 96 h after onset of standing heat was lower than in AA sows ($P < 0.05$). No significant correlation between embryo survival and progesterone concentration at 36, 48, 72, and 96 h after onset of standing heat was observed ($P > 0.05$). However, there was an association between maximum, mean, and minimum LH concentrations on d 28 after weaning and weaning-to-estrus interval ($r = -0.51$ to -0.54, $P < 0.01$).

DISCUSSION

Consistent with the data reported by Zak et al. (1997a), the different feeding regimens caused differential sow body weight changes. The body weight loss of 30.0 and 30.4 kg in AR and AR + GnRH sows were also similar to that seen in similarly restricted AR sows in a previous study (Chapter 3). However, in the present experiment, the AA, AR, and AR + GnRH sows lost more body weight during lactation than the comparable AA and AR sows in the study of Zak et al. (1997a). Consistent with the data reported by Pond and Mersmann (1988) and by King and Williams (1984), pigs suckling sows fed to appetite during lactation grew faster than the pigs suckling sows whose feed intake was restricted. Although pig growth rate over the first 3 wk of lactation was not different among treatments, growth rate of pigs suckling AA sows over the last week of lactation was higher than that of pigs suckling AR and AR + GnRH sows. Thus, as pointed out by Fahmy and Dufour (1976), reducing feed intake during the last week of lactation compromises milk yield and hence pig growth rate. The lack of an effect of similar pattern of feed restriction on pig growth rate in the study of Zak et al. (1997a) is likely due to the lower number of six pigs suckling in that experiment. The difference in suckling pig number could be the cause of different pig growth rate, since sows nursing their young are subjected to catabolism which is proportionate to the number of young suckling pigs (Fahmy and Dufour, 1976). The endocrine responses to treatment were consistent with previous results that feed restriction reduced plasma insulin (Mullan and Close, 1991; Koketsu et al., 1996; Zak et al., 1997a) and IGF-I concentrations (Zak et al., 1997a). It has been suggested that leptin is a metabolic signal to the reproductive system

(Barash et al., 1996). This experiment showed that feed restriction for 7 d depressed postprandial plasma leptin concentrations in lactating sows. The decreased leptin could act at both the ovarian level and on the hypothalamic-pituitary axis to regulate the reproductive system. The data of Zachow and Magoffin (1997), showing the presence of functionally active leptin receptors in rat ovarian cells, raised the possibility that leptin could influence ovarian function locally. On the contrary, the presence of leptin receptors in the hypothalamus (Zamorano et al., 1997), and the fact that exogenous leptin increased basal LH levels and stimulated follicular development in feed restricted mice (Barash et al., 1996), suggested that leptin acts at a central level. Carro et al. (1997) treated rats with leptin antiserum and found a marked impairment in LH pulsatility. The significant correlation between plasma leptin and mean LH concentrations in the present experiment supported the hypothesis that leptin acts at a central level. In recent clinical studies, plasma leptin levels were considered to reflect changes in total adipose mass, rather than meal consumption or dietary energy source (Weigle et al., 1997). In the current experiment, AR and AR + GnRH sows lost more body weight than AA sows during lactation, but there was no difference in backfat loss among the three groups. Backfat change may, therefore, be a poor indicator of sow metabolic status, whereas feed restriction resulted in changes in plasma leptin concentration. If leptin concentrations are assumed to reflect changes in adipose tissue stores and mobilization, this suggests that measurement of leptin is a much more sensitive measure of "adiposity" than backfat determinations. Alternatively, in this experimental paradigm, leptin may respond to short-term changes in energy or other dietary intakes.

The suckling stimulus is the predominant inhibitor of LH secretion in the lactating sow (Foxcroft, 1992), but there is also extensive evidence for nutritional effects on LH secretion during lactation. Mullan et al. (1991) reported that lower energy intakes and larger litter size resulted in increased tissue catabolism, with lower plasma insulin and suppressed episodic LH secretion. Zak et al. (1997a) further examined the effect of feed intake on LH secretion in an experimental paradigm that involved three patterns of feeding during lactation in primiparous sows suckling only six pigs. Notwithstanding the dominant inhibitory effect of suckling, differences in the pattern of feed intake during lactation again produced significant effects on episodic LH secretion during lactation and

on sow post-weaning fertility, although there was little influence of previous treatment on the very robust LH response to weaning. Similar interrelationships between feed intake, sow metabolic state and LH secretion during lactation were also reported by Quesnel et al. (1998). The present studies further confirm that feed restriction inhibits episodic LH secretion in the lactating sow.

The studies of Sesti and Britt (1993, 1994) provided the most direct evidence that reduced GnRH secretion from the hypothalamus is likely the primary cause of a reduction in pituitary LH secretion in the lactating sow. Increased follicular development in response to exogenous GnRH treatment during lactation (Cox and Britt, 1982; Rojanasthien et al., 1987; De Rensis et al., 1991) is consistent with this concept. The extensive studies by Booth (1990) on the endocrine mechanisms mediating effects of nutrient intake on ovarian function in prepubertal gilts also suggested that the dynamic changes in gonadotropin secretion immediately before recruitment of follicles into the follicular phase could be a critical determinant of subsequent fertility. Therefore, the main objective of the current experiment was to determine the extent to which stimulation of endogenous LH pulsatility in primiparous sows undergoing feed restriction would overcome the primary, central effects of feed restriction on the reproductive axis. Exogenous GnRH infusion every 6 h restored the LH secretion in AR + GnRH sows to that seen in AA sows, confirming the ability of the pituitary gland in feed restricted lactating sows to respond to exogenous GnRH treatment and the assumption that feed restriction acts primarily at the hypothalamic level to inhibit LH secretion (I'Anson et al., 1991; Foxcroft et al., 1995). After weaning, plasma LH secretion in both AA and AR sows, and FSH secretion in AR sows, showed a substantial increase, consistent with the data of Zak et al. (1997a). However, there was no LH or FSH response to weaning in AR + GnRH sows. One possible explanation for AR + GnRH sows not showing a response to weaning is that GnRH stimulation in the presence of feed restriction limited the readily releasable pools of LH at the time of weaning. Another possible explanation for the lack of an LH response to weaning is that GnRH treatment during the last week of lactation down-regulated the number of GnRH receptors in the pituitary. Although no studies have been performed in feed restricted lactating sows to investigate the number of GnRH receptors in the pituitary, Wu et al. (1994) demonstrated that in vitro treatment of sheep

pituitary cells with a GnRH agonist down-regulated the number of GnRH receptors. In both situations, an increase in pulsatile GnRH secretion after weaning might not be associated with a corresponding increase in episodic LH secretion. The presence of some episodic LH release in the AR + GnRH sows after weaning suggests, however, that a latent effect of GnRH treatment on the GnRH pulse generator was the primary reason for these sows failing to show the usual LH response to weaning. This would also explain the lack of an increase in FSH concentrations in AR + GnRH sows after weaning. Whatever the mechanism, GnRH treatment during lactation in the feed restricted sows suppressed the expected LH and FSH responses to weaning and this could well have compromised the possible beneficial effect of GnRH treatment on subsequent fertility. It has been suggested previously that after low levels of feed intake during lactation, reduced embryo survival in the weaned sow may be related to reduced gonadotropin secretion in the post-weaning period (Edwards and Foxcroft, 1983; Kirkwood et al., 1987a,b; Parr et al., 1993).

Progesterone concentration at 96 h after onset of standing heat was lower in AR than that in AA sows, even though ovulation rate did not differ between these groups. This difference could therefore be related to lower luteal function in AR sows and is consistent with results of previous studies suggesting that differences in progesterone concentrations may mediate latent effects of lactational catabolism on postweaning fertility (Kirkwood et al., 1987b; Clowes et al., 1994; Jindal et al., 1996). The lack of a difference in progesterone concentrations between AA and AR + GnRH sows raises the possibility that GnRH treatment had beneficial effects on luteal function. There was no difference in ovulation rate and embryo survival rate among the three treatment groups. These results conflict with the previous data of Zak et al. (1997a). This may be attributable to the somewhat different models used, such that litter size, feed intake, and their interactive effects on energy balance and endocrine changes may have determined different effects on ovulation rate and embryo survival. Compared to the AA and AR sows in the experiment of Zak et al. (1997a), in the current experiment the ovulation rate in AA sows was lower (17.7 vs 19.9), and in AR sows was higher (16.7 vs 15.4). The increase in litter size was a factor that may also have influenced LH secretion, as noted by Mullan et al. (1991). Suckling more pigs caused the sows to be more catabolic during

lactation. As suggested by Brooks (1982), sows that become catabolic during lactation may remain so after weaning and as a consequence have reduced ovulation rate. On the other hand, the feed intake in AR sows during the last week of lactation was higher than that of previous AR sows (2.7 vs 2.1 kg, Zak et al., 1997a). In addition, feed intake between weaning and mating in AR and AR + GnRH sows was higher than AA sows and increased feed intake may have a beneficial effect on ovulation rate as observed by King and Williams (1984). Possibly, for the same reason, the more catabolic status during lactation in AA sows suckling more pigs, could contribute to the observed low embryo survival rate in these animals (67.7% compared to 87.5% in the AA sows of Zak et al., 1997a). Embryo survival in AR sows was comparable to that of the AR sows as observed by Zak et al. (1997a), and consistent with the data we previously reported (Chapter 3). Even if a normal LH and FSH response to weaning had occurred after exogenous GnRH treatment in AR + GnRH sows, nutritional effects at the ovarian level more still have limited reproductive performance. Local factors such as insulin, IGF-I, and leptin play a role in determining reproductive performance in feed-restricted animals (I'Anson et al., 1991; Foxcroft, 1992; Barash et al., 1996). Effects of insulin on follicular development and ovulation rate have been reported by Cox et al. (1987) and a streptozotocin-induced diabetic gilt model was used by Meurer et al. (1991) to investigate the role of insulin in recruitment of ovulatory follicles. The number of follicles larger than 3 mm in diameter, recovered 75 h after PMSG treatment, was similar in both diabetic and insulin-treated diabetic animals; however, the number of atretic follicles in diabetic gilts was greater than in insulin-treated animals. These differences in follicular development were not associated with changes in gonadotropin concentrations. Similarly, in a progestagen-treated prepubertal gilt model (Cosgrove et al., 1992), 5 d of realimentation affected follicular development, independent of changes in LH secretion.

In conclusion, this experiment confirmed that feed restriction during lactation affected gonadotropin secretion. Although, GnRH therapy in feed restricted sows restored LH secretion to that seen in unrestricted animals, it did not improve overall reproductive performance. This may have been due to the paradoxical effect of GnRH treatment in lactation suppressing the LH and FSH responses to weaning. As a result, GnRH treatment in lactation does not appear to be effective in alleviating a metabolically induced

reduction in sow fertility. However, as there was a significant correlation between post-weaning maximum, mean, and minimum LH concentrations and weaning-to-estrus interval, an appropriate pattern of LH secretion may still be important for sow fertility.

LITERATURE CITED

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996; 137: 3144-3147.

Booth PJ. Metabolic influences on hypothalamic-pituitary-ovarian function in the pig. J Reprod Fertil Suppl 1990; 40: 89-100.

Booth PJ, Craigon J, Foxcroft GR. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J Anim Sci 1994; 72: 2415-2424.

Brooks PH. The gilt for breeding and for meat. In: Cole DJA, Foxcroft GR (eds.), Control of Pig Reproduction. Butterworths, London. 1982; 211-224.

Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, Dieguez C. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology 1997; 66: 375-3777.

Clowes EJ, Aherne FX, Foxcroft GR. Effect of delayed breeding on the endocrinology and fecundity of sows. J Anim Sci 1994; 72: 283-291.

Cosgrove JR, Booth PJ, Foxcroft GR. Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. J Reprod Fertil 1991; 91: 277-284.

Cosgrove JR, Foxcroft GR. Nutrition and reproduction in the pig: ovarian aetiology. Proc. of 13th International Conference on Animal Reproduction, Sydney, Australia. Anim Reprod Sci 1996; 42: 131-141.

Cosgrove JR, Tilton JE, Hunter MG, Foxcroft GR. Gonadotropin-independent mechanisms participate in ovarian responses to realimentation in feed-restricted prepubertal gilts. Biol Reprod 1992; 47: 736-745.

Cosgrove JR, Urbanski HF, Foxcroft GR. Maturational changes in gonadotrophin secretion: The LH response to realimentation and a nocturnal increment in LH secretion of feed-restricted prepubertal gilts. J Reprod Fertil 1993; 98: 293-300.

Cox NM, Britt JH. Pulsatile administration of gonadotropin releasing hormone to lactating sows: Endocrine changes associated with induction of fertile estrus. Biol Reprod 1982; 27: 1126-1137.

Cox NM, Stuart MJ, Althen TG, Bennett WA, Miller HW. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J Anim Sci 1987; 64: 507-516.

De Rensis F, Hunter MG, Grant SA, Lancaster RT, Foxcroft GR. Effect of estrogen administration on endogenous and luteinizing hormone-releasing hormone-induced

luteinizing hormone secretion and follicular development in the lactating sow. Biol Reprod 1991; 44: 975-982.

Edwards S, Foxcroft GR. Endocrine changes in sows weaned at two stages of lactation. J Reprod Fertil 1983; 67: 161-172.

Fahmy MH, Dufour JJ. Effects of post-weaning stress and feeding management on return to estrus and reproductive traits during early pregnancy in swine. Anim Prod 1976; 23: 103-110.

Foxcroft GR. Nutritional and lactational regulation of fertility in sows. J Reprod Fertil Suppl 1992; 45: 113-125.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Foxcroft GR, Aherne FX, Clowes EJ, Miller HM, Zak LJ. Sow fertility: The role of suckling inhibition and metabolic status. In: Ivan M (ed.), Animal Science Research and Development: Moving Towards a New Century. Ministry of Supply and Services Canada; 1995; Ottawa, Canada. 377-391.

I'Anson H, Foster DL, Foxcroft GR, Booth PJ. Nutrition and reproduction. Oxford Rev Reprod Biol 1991; 13: 239-311.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. J Anim Sci 1996; 74: 620-624.

King RH, Williams IH. The effect of nutrition on the reproductive performance of first-litter sows. 1. Feeding level during lactation and between weaning and remating. Anim Prod 1984; 38: 241-247.

Kirkwood RN, Baidoo SK, Aherne FX, Sather AP. The influence of feeding level during lactation on the occurrence and endocrinology of the postweaning estrus in sows. Can J Anim Sci 1987a; 67: 405-415.

Kirkwood RN, Lythgoe ES, Aherne FX. Effect of lactation feed intake and gonadotrophin-releasing hormone on the reproductive performance of sows. Can J Anim Sci 1987b; 67: 715-719.

Koketsu Y, Dial GD, Pettigrew JE, Marsh WE, King VL. Influence of imposed feed intake patterns during lactation on reproductive performance and on circulating levels of glucose, insulin and luteinizing hormone in primiparous sows. J Anim Sci 1996; 74: 1036-1046.

Meurer KA, Cox NM, Matamoros IA, Tubbs RC. Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J Reprod Fertil 1991; 91: 187-196.

Mullan BP, Close WH. Metabolic and endocrine changes during the reproductive cycle of the sow. In: E.S. Batterham (ed.), Manipulating Pig Production III. Australasian Pig Science Association, Victoria, Australia. 1991; 32 (Abstract).

Mullan BP, Close WH, Foxcroft GR. Metabolic state of the lactating sow influences plasma LH and FSH before and after weaning. In: E.S. Batterham (ed.), Manipulating Pig Production III. Australian Pig Science Association, Victoria, Australia. 1991; 31 (Abstract).

NRC. Nutrient Requirements of Swine (9th ed.). National Academy Press, Washington, DC. 1988

Parr RA, Hughes PE, Davis IF, Miles MA, Ferlazzo JC. Nutrition, progesterone and the early pig embryo. Proc 4th Int Conf Pig Reprod; Columbia. 1993; 93.

Pond WG, Mersmann HJ. Comparative response of lean or genetically obese swine and their progeny to severe feed restriction during gestation. J Nutr 1988; 118: 1223-1231.

Quesnel H, Pasquier A, Mounier AM, Prunier A. Influence of feed restriction during lactation on gonadotropin hormones and ovarian development in primiparous sows. J Anim Sci 1998; 76: 856-863.

Quesnel H, Prunier A. Endocrine bases of lactational anoestrus in the sow. Reprod Nutr Dev 1995; 35: 395-414.

Rojanasthien SAM, Lundeheim N, Einarsson S. Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim Reprod Sci 1987; 13: 299-307.

SAS. SAS User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC. 1990.

Sesti, LAC, Britt JH. Relationship of secretion of GnRH in vitro to changes in pituitary concentrations of LH and FSH and serum concentrations of LH during lactation in sows. J Reprod Fertil 1993; 98: 393-400.

Sesti LAC, Britt JH. Secretion of gonadotropins and estimated releasable pools of gonadotropin-releasing hormone and gonadotropins during establishment of suckling-induced inhibition of gonadotropin secretion in the sow. Biol Reprod 1994; 50: 1078-1086.

Shaw HJ, Foxcroft GR. Relationships between LH, FSH and prolactin secretion and reproductive activity in the weaned sow. J Reprod Fertil 1985; 75: 17-28.

Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab 1997; 82: 561-565.

Wu JC, Sealfon SC, Miller WL. Gonadal hormones and gonadotropin-releasing hormone (GnRH) alter messenger ribonucleic acid levels for GnRH receptors in sheep. Endocrinology 1994; 134: 1846-1850.

Zachow RJ, Magoffin DA. Direct intraovarian effects of leptin: Impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17 beta production by rat ovarian granulosa cells. Endocrinology 1997; 138: 847-850.

Zak LJ, Cosgrove JR, Aherne FX, Foxcroft GR. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J Anim Sci 1997a; 75: 208-216.

Zak LJ, Xu X, Hardin RT, Foxcroft GR. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J Reprod Fertil 1997b; 110: 99-106.

Zamorano PL, Mahesh VB, De Sevilla LM, Chorich LP, Bhat GK, Brann DW. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology 1997; 65: 223-228.

Table 4.1. Composition of experimental diets as formulated

Item	Lactating sow	Gestating sow
Ingredient, %		
Barley	79.4	85.8
Soybean meal	12.5	4.4
Canola	--	4.3
Lysine	.2	--
Limestone	1.4	1.5
Dicalcium phosphate	2	1.4
Vitamin-mineral premix[a]	1	1
Iodized salt	.5	.6
Oil	3	1
Chemical analysis		
Metabolizable energy, kcal/kg	3,200	3,000
Crude protein, %	15.86	13.75
Lysine, %	.86	.56
Ca, %	1	.93
P, %	.83	.7

[a]: Supplied the following per kilogram of diet: 10,000 IU vitamin A, 1,000 IU vitamin D, 80 IU vitamin E, 2 mg vitamin K, 30 µg vitamin B_{12}, 12 mg riboflavin, 25 mg niacin, 25 mg calcium pantothenate, 1,000 mg choline chloride, 200 µg biotin, 200 mg folic acid, 5 mg ethoxyquin, 150 mg iron, 12 mg manganese, 120 mg zine, 12 mg copper, 200 µg iodine and 100 µg selenium.

Table 4.2. LSMeans (± SEM) of feed intake, sow body weight, body weight change, backfat thickness, backfat change, and litter weight at weekly intervals during lactation in AA (n=8), AR (n=12), and AR + GnRH (n=12) sows

	Treatment		
Item	AA	AR	AR + GnRH
Feed intake, kg			
Week 1	4.2 ± 0.3	4.6 ± 0.2	4.8 ± 0.2
Week 2	4.8 ± 0.3	4.8 ± 0.2	5.4 ± 0.2
Week 3	4.8 ± 0.2	4.9 ± 0.2	5.4 ± 0.2
Week 4	5.8 ± 0.1[a]	2.7 ± 0.1[b]	2.7 ± 0.1[b]
Weaning to mating	2.9 ± 0.3[a]	4.1 ± 0.2[b]	3.9 ± 0.2[b]
Body weight, kg			
Farrowing	189.3 ± 5.6	196.7 ± 4.6	197.1 ± 4.6
D 7	184.0 ± 5.8	194.9 ± 4.8	194.7 ± 4.8
D 14	178.1 ± 5.7	190.0 ± 4.7	191.2 ± 4.7
D 21	171.9 ± 5.7	183.9 ± 4.7	185.0 ± 4.7
D 28	169.9 ± 5.8	166.8 ± 4.7	167.8 ± 4.7
Body weight change, kg, d 1 to 28	-19.4 ± 3.2[a]	-30.0 ± 2.6[b]	-30.4 ± 2.6[b]
Backfat thickness, mm			
Farrowing	16.2 ± 0.8	17.2 ± 0.6	16.1 ± 0.6
D 7	16.0 ± 0.8	16.4 ± 0.7	15.7 ± 0.7
D 14	14.7 ± 0.8	15.8 ± 0.7	15.2 ± 0.7
D 21	13.7 ± 0.8	14.5 ± 0.7	13.8 ± 0.7
D 28	12.8 ± 0.9	13.3 ± 0.7	12.4 ± 0.7
Backfat change, mm, d 1 to 28	-3.4 ± 0.6	-3.9 ± 0.5	-3.9 ± 0.5
Energy balance, based on the data above, kcal ME/d			
Week 1-3	-5,745 ± 922	-4,198 ± 751	-3,637 ± 751
Week 4	-2,676 ± 1,050[a]	-9,308 ± 855[b]	-10,533 ± 855[b]
Litter size	9.2 ± 0.2	9.2 ± 0.2	9.6 ± 0.2
Pig growth rate, g/d			
Week 1-3	258.0 ± 10.9	239.6 ± 8.9	243 ± 8.9
Week 4	276.5 ± 12.8[a]	216.6 ± 10.4[b]	226.4 ± 10.4[b]
Week 1-4	262.5 ± 8.3[a]	234.4 ± 6.7[b]	239.6 ± 6.8[b]

[a,b] Within a row, means lacking common letter differ ($P < 0.05$).

Table 4.3. LSMeans (± SEM) of plasma IGF-I, pre- and post-prandial insulin concentrations and LH pulse frequency (per 12 h period) on d 21, before and after weaning on d 28 of lactation

Item	Treatment		
	AA (n = 7)	AR (n = 10)	AR + GnRH (n = 12)
IGF-I, ng/ml			
D 21	75.9 ± 5.5	80.2 ± 6.6^{x}	86.0 ± 7.7^{x}
D 28	80.2 ± 6.8^{a}	50.7 ± 5.8^{by}	54.5 ± 4.7^{by}
After weaning	76.6 ± 7.3^{a}	52.9 ± 6.0^{by}	52.9 ± 5.0^{by}
Insulin, ng/ml			
Preprandial d 21	1.1 ± 0.3^{x}	1.2 ± 0.2^{x}	1.0 ± 0.2^{x}
Prandial/Postprandial d 21	2.8 ± 0.2^{y}	3.0 ± 0.2^{y}	3.0 ± 0.2^{y}
Preprandial d 28	1.3 ± 0.2^{x}	1.1 ± 0.1^{x}	0.8 ± 0.1^{x}
Prandial/Postprandial d 28	2.7 ± 0.2^{ay}	1.8 ± 0.1^{bx}	1.4 ± 0.1^{bx}
After weaning	1.5 ± 0.2^{x}	1.5 ± 0.2^{x}	1.3 ± 0.2^{x}
LH frequency per 12 h			
D 21	2.9 ± 1.0	2.9 ± 0.6^{x}	3.2 ± 0.5
D 28 Pre-weaning	2.2 ± 0.5^{a}	0.5 ± 0.2^{by}	2.5 ± 0^{a}
Post-weaning	High *	High	2.7 ± 0.5

^{a,b}Within a horizontal row, means lacking common superscript letter differ ($P < 0.05$).

^{x,y}Within a column, means lacking common superscript letter differ ($P < 0.05$).

*: High = objective estimates not available, but very high pulse frequency assumed (see Figure 4.3).

Table 4.4. LSMeans (± SEM) of weaning-to-estrus interval, ovulation rate, number of viable embryos, embryo survival rate, and embryo crown-rump length in AA, AR and AR + GnRH sows (for full definition see text)

Item	Treatment		
	AA (n = 8)	AR (n = 12)	AR + GnRH (n = 11)
Weaning-to-estrus interval, h	110.0 ± 4.6[a]	121.6 ± 3.8[b]	125.6 ± 3.8[b] (n = 12)
Pregnancy stage*, d	28.4 ± 0.4	28.3 ± 0.4	28.2 ± 0.4
Ovulation rate	17.7 ±1.0	16.7 ± 0.8	17.5 ± 0.9
Number of viable embryos	12.2 ± 1.5	12.4 ± 1.2	13.2 ± 1.3
Embryonic survival, %	67.7 ± 6.5	73.2 ± 5.3	75.4 ± 5.5
Crown-rump length, mm	24.1 ± 0.5	23.3 ± 0.4	23.6 ± 0.4

[a,b]Within a row, means lacking common superscript letter differ ($P < 0.05$)

*: The first 24-h period after onset of estrus was designated as d 0 of pregnancy.

Table 4.5. LSMeans (± SEM) of plasma progesterone concentrations at 36, 48, 72, and 96 h after onset of standing heat in AA, AR, and AR + GnRH sows

	Treatment		
Time	AA (n = 8)	AR (n = 12)	AR + GnRH (n = 12)
36	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
48	0.9 ± 0.1	0.7 ± 0.1	1.0 ± 0.2
72	3.6 ± 0.5	2.9 ± 0.4	3.1 ± 0.5
96	7.1 ± 0.9[a]	5.0 ± 0.4[b]	6.5 ± 0.8[ab]

[a,b]Within a row, means lacking common superscript letter differ ($P < 0.05$)

Figure 4.1. Plasma leptin concentrations on d 21 in all sows (n = 29), d 28 of lactation and the day after weaning before feeding in AA (n = 7) and pooled AR and AR + GnRH sows (n = 22) (↓ indicates time of feeding; * indicates increase in leptin level after feeding compared to preprandial period, $P < .0001$; x and y indicate difference between groups in the period after feeding, $P < .001$).

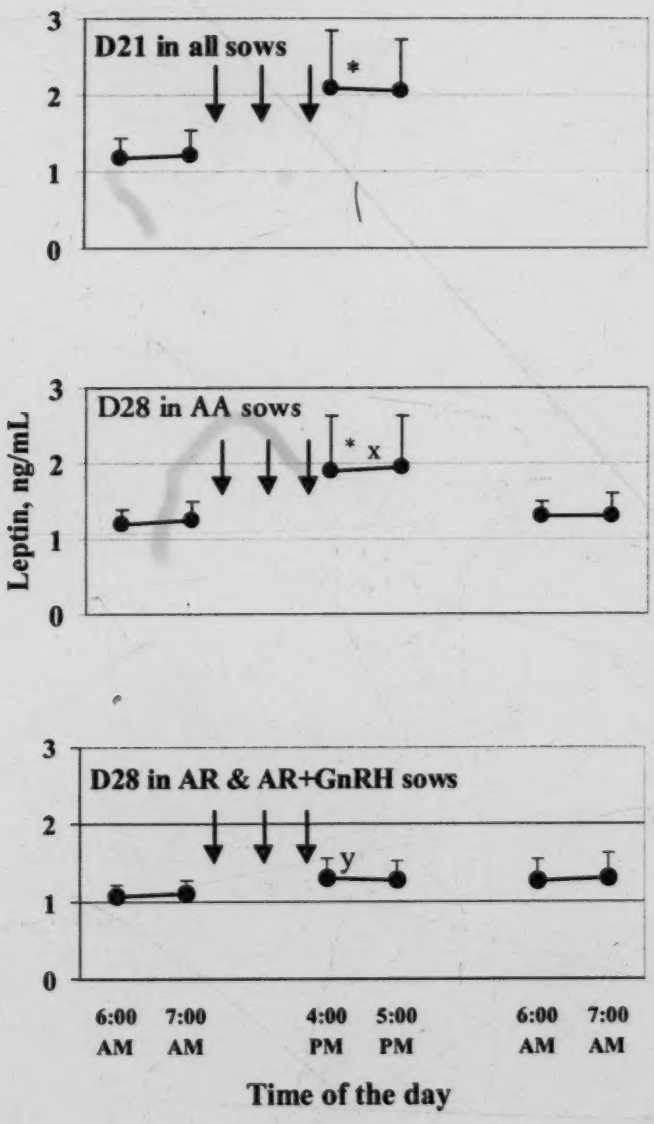

D21 in all sows
*
D28 in AA sows
* x
D28 in AR & AR+GnRH sows
y
3
2
1
0
Leptin, ng/mL
6:00 AM
7:00 AM
4:00 PM
5:00 PM
6:00 AM
7:00 AM
Time of the day

Figure 4.2. Plasma LH maximum, mean, and minimum concentrations and FSH concentrations on d 21 and 28 before and after weaning in AA, AR, and AR + GnRH sows.

a, b: The difference between treatment groups within day.

x, y, z: The difference between days within treatment.

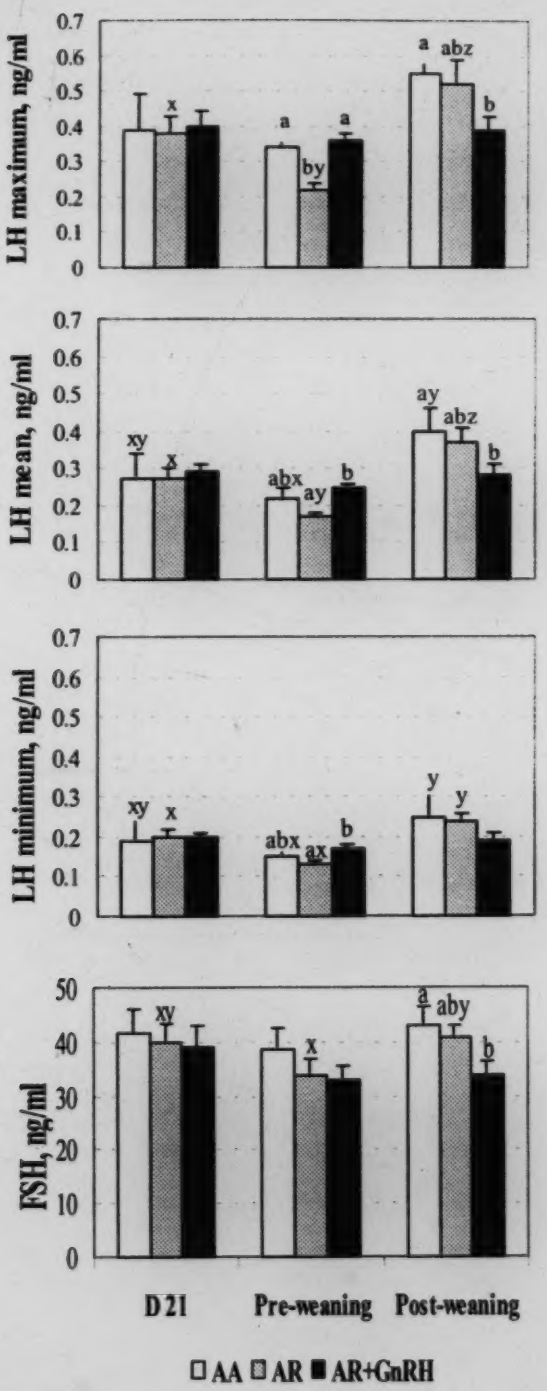
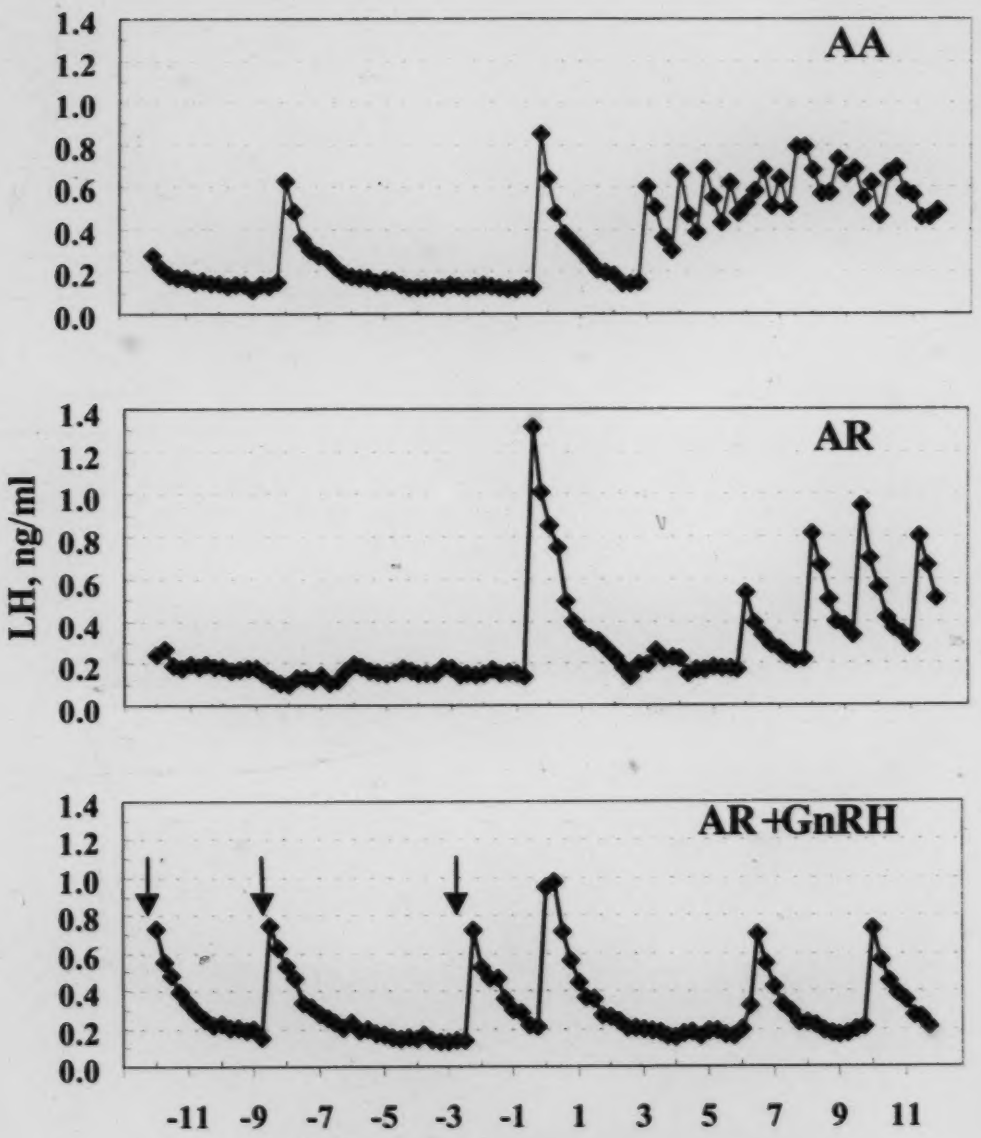

LH maximum, ng/ml
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
x
a
by
a
a
abz
b
LH mean, ng/ml
xy
x
abx
ay
b
ay
abz
b
LH minimum, ng/ml
xy
x
abx
ax
b
y
y
FSH, ng/ml
50
40
30
20
10
0
xy
x
a
aby
b
D 21
Pre-weaning
Post-weaning
AA AR AR+GnRH

Figure 4.3. LH profiles representing AA, AR, and AR + GnRH sows on d 28 before, and after, weaning (0 is weaning time). ↓ indicates the time of bolus GnRH injection *i.v.*


AA
AR
AR+GnRH
LH, ng/ml
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-11 -9 -7 -5 -3 -1 1 3 5 7 9 11
Time before and after weaning, h

CHAPTER 5

LUTEAL FUNCTION IN THE IMMEDIATE POST-OVULATORY PERIOD IN FEED-RESTRICTED AND INSULIN-TREATED PIGS: PROGESTERONE PRODUCTION *IN VITRO* AND mRNA EXPRESSION FOR KEY STEROIDOGENIC ENZYMES

INTRODUCTION

Suboptimal luteal function has been suggested as a major cause of infertility and studies in pigs suggest that differences in progesterone production in early pregnancy mediate nutritional effects on embryonic survival (Hunter et al., 1996; Foxcroft et al., 1997; van den Brand et al., 2000). An earlier rise in plasma progesterone concentrations after the LH surge in more prolific Chinese Meishan compared to Large White gilts has also been observed and may play a key role in the superior embryonic survival reported in the Meishan breed (Hunter et al., 1996). Although data presented in Chapter 3 showed no beneficial effects of exogenous progesterone treatment on embryonic survival in primiparous sows, progesterone supplementation in gilts in early pregnancy did enhance embryonic survival at d 30 of pregnancy (Ashworth, 1991; Jindal et al., 1997). In a previous experiment, that formed the basis for the present study, feed restriction in gilts during the second week of the estrous cycle depressed circulating progesterone concentrations at 48 and 72 h after onset of estrus and decreased embryonic survival rate at d 28 of pregnancy, compared with non-restricted gilts or gilts feed-restricted during the first week of the cycle (Almeida et al. 2000a). Using an extension of this experimental model (Almeida et al., 2000b), again showed negative effects of feed restriction during the second week of the cycle on plasma progesterone concentrations in early pregnancy. Furthermore, insulin treatment during feed restriction was able to counteract negative effects on progesterone. Since differences in metabolism and clearance of progesterone were unlikely to contribute to differences in plasma progesterone concentrations in this

---

Ψ: As part of this major collaborative study, work on endocrine responses to feed restriction and insulin treatment and effects on embryonic development has been submitted to the Journal of Animal Science by Almeida FRCL, Mao J, Novak S, Cosgrove JR and Foxcroft GR; Work presented in Chapter 5 has been submitted to Biology of Reproduction by Mao J, Treacy BK, Almeida FRCL, Novak S, Dixon WT and Foxcroft GR.

experimental model, we hypothesized that the differences in progesterone concentrations were likely due to differences in luteal function.

Early corpus luteum development in the pig is characterized by reduced numbers of low affinity LH receptors on luteal cells, compared to either granulosa cells before ovulation or luteal cells in the mid-luteal phase (Gebarowska et al., 1997). Therefore, low progesterone concentrations and a delayed rise in progesterone in feed-restricted gilts may be a consequence of low sensitivity of the corpus luteum to LH. As steroidogenic acute regulatory (StAR) protein, and the cholesterol side-chain cleavage (P450scc) and 3beta-hydroxysteroid-dehydrogenase (3beta-HSD) enzymes are essential to normal luteal function (Smith et al., 1994; Carr, 1998), and StAR protein is a key regulator of progesterone synthesis (Orly and Stocco, 1999), differences in luteal function may also be related to differences in StAR, P450scc, or 3beta-HSD mRNA expression. Finally, intraovarian growth factors, such as insulin-like growth factor I (IGF-I), fibroblast growth factor (FGF), and transforming growth factor β (TGFβ) modulate the response of the corpus luteum to circulating gonadotropins (Adashi and Roban, 1992) and may exert an additional level of control on luteal function. In turn, the availability of such growth factors is influenced by the activity of metalloproteinases (MMP) (IGF-I: Urban et al., 1990; Caubo et al., 1989; FGF: Biswas et al, 1988; TGFβ: Kubota et al., 1994). Given that plasma progesterone concentration appears to increase earlier in Chinese Meishan, compared to Large White pigs, the report of higher gelatinase activity (MMP-2 and MMP-9) and lower tissue inhibitors of metalloproteinase (TIMP-1 and TIMP-2) in the luteinizing follicles of prolific Meishan females (Driancourt et al., 1998), suggests that differences in TIMP-MMP-growth factor activity may also regulate progesterone production.

Insulin has been identified as an important modulator of reproductive function. Insulin treatment increased follicular fluid steroids (Whitley et al., 1998a), the number of large follicles (Whitley et al., 1998b), and farrowing rate and litter size (Ramirez et al., 1997) in weaned sows. In addition, supplementary insulin increased ovulation rate (Cox et al., 1987) and decreased follicular atresia (Matamoros et al., 1990, 1991) in cyclic gilts when given during the preovulatory period. Exogenous insulin restores follicular growth in nutritionally anestrous gilts (Britt et al., 1988), and feeding additional dietary energy

increased ovulation rate and insulin concentrations (Flowers et al., 1989; Beltranena et al., 1991). Therefore, insulin treatment in the feed-restricted gilts may counteract the effect of feed restriction by affecting folliculogenesis and subsequent luteal function.

Therefore, as part of a major collaborative study, the objectives of the present experiment were to use our established feed-restricted cyclic gilt model to investigate luteal function in the immediate post-ovulatory period in feed-restricted and insulin-treated gilts by determining: 1) progesterone production and release by minced luteal tissue and by dispersed luteal cells *in vitro*; 2) responsiveness of luteal tissue and cells to LH stimulation; 3) mRNA expression for StAR protein, P450scc and 3beta-HSD enzymes; 4) mRNA expression for matrix metalloproteinases, MMP-2, MMP-9, and their inhibitors, TIMP-1, and TIMP-2 in luteal tissue; and 5) associations among these parameters and the relationship between these characteristics and oviductal and peripheral plasma progesterone concentrations. The results of this study provide some of the first evidence for effects of nutritional and metabolic status during early follicular development on subsequent luteal function.

MATERIALS AND METHODS

Animals and Treatments

The research protocol involving animal use and care was performed in accordance with the Canadian Council on Animal Care Guidelines and with the approval of the University Animal Care and Use Committee. Full details of the management, nutrition and other treatments of the gilts used in this experiment were presented in the paper of Almeida et al. (2000b). Briefly, littermate Camborough × Canabrid cyclic gilts in second estrus (Pig Improvement [Canada] Ltd, Acme, AB, Canada) were selected from 15 litters, and allocated within litter to one of three treatments. RH gilts (n=14) were feed restricted at approximately 75% of ad libitum intake (2.1 × energy maintenance requirements) from d 1 to 7 of the second estrous cycle and then fed approximately 95% of ad libitum intake (2.8 × maintenance) from d 8 of the cycle until estrus. Their littermates were fed 2.8 × maintenance from d 1 to 7, restricted to 2.1 × maintenance from d 8 to 15, with (HR+I, n=15) or without (HR, n=15) long-acting insulin injection at the time of feeding (0.8 I.U./kg body wt/d, Lente Iletin II; Eli Lilly, Indianapolis, IN), and then returned to 2.8 ×

maintenance until estrus. Animals were checked for estrus every 6 h from d 18 of the treatment cycle and inseminated 12 and 24 h after onset of estrus. From 24 h after onset of estrus, ovulation was monitored every 6 h by real time ultrasound (Pie Medical Scanner 200, Can Medical, Kingston, ON). By 12 to 24 h after ovulation, the ovary with the most corpora lutea was surgically removed by hemiovariectomy and immediately transported on ice to the laboratory in 20 ml Hank's balanced salt solution (HBSS, Sigma, St. Louis, MO) without calcium and magnesium, at pH 7.4. Corpora lutea were dissected from the ovary and 250 to 300 mg of luteal tissue, pooled from all corpora lutea, was snap frozen in liquid nitrogen and stored at -80°C for later mRNA extraction and analysis. The remaining luteal tissue was prepared for tissue/cell culture. The interval between surgery and dissection was always less than 20 min.

Incubations

Luteal tissue fragments

The pooled luteal tissue obtained from each gilt was finely minced on ice with two scalpel blades and washed twice in 50 ml HBSS without calcium and magnesium. Based on the method described by Hunter (1981), known amounts of minced tissue (20 ± 2 mg) were placed into 24 culture tubes. Samples in four tubes were frozen immediately for assay of tissue progesterone content before culture (T0 P4). Minced tissue in the remaining tubes was cultured in 2 ml Eagle's minimum essential medium (MEM) (Sigma, St. Louis, MO) containing 0, 0.1, 1, 10, and 100 ng pLH/ml (kindly provided by NIDDK's National Hormone and Pituitary Program and Dr. A.F. Parlow, Harbor-UCLA Medical Center, CA), with four replicates per dose. LH activity in the medium were confirmed by RIA. After gassing with 95% (v/v) O_2 / 5% (v/v) CO_2 for 10 seconds, the tubes were capped and incubated in a shaking water bath for 3 h at 37°C. At the end of incubation, tissue and medium were separated by centrifugation at 500 × g for 5 min and the tissue subsequently homogenized (Polytron, Janke & Kunkel IKA-Labortechnik, Germany) in absolute alcohol (HPLC grade, Fisher Scientific Ltd., Nepean, ON). The ethanol extract and the medium were assayed for progesterone as described below.

Dispersed cells

The remaining luteal tissue was washed and subjected to enzyme dissociation as described by Gadsby et al. (1996). Briefly, luteal tissue was incubated with 0.1% (w/v) collagenase (type I, Sigma, St. Louis, MO) and 0.002% (w/v) DNase (Type I, Boehringer Mannheim Canada, Laval, QC) in HBSS at a volume of 10 ml/g tissue at 37°C for 70 min while being agitated by a magnetic stirrer. Undigested tissue fragments were removed by a brief centrifugation step. The remaining cell suspension was filtered through an 80 µm nylon mesh, washed twice with fresh MEM (15 ml), and cell number was then counted twice with a hemocytometer. 100,000 viable cells were incubated in 1 ml MEM per tube in quadruplicate at the various LH concentrations described above. Viability, estimated by the 0.2% (w/v) Trypan blue (Sigma, St. Louis, MO) exclusion test (Hunter, 1981), was greater than 80% before incubation and greater than 70% after the 3-h incubation. Four aliquots of the cell suspension were immediately frozen for subsequent estimation of the steroid in the medium before incubation. All steps were carried out at 4°C unless otherwise indicated.

Progesterone Radioimmunoassay

Peripheral and Oviductal Progesterone RIA

At surgery, peripheral blood samples were collected by jugular venipuncture and oviductal blood samples were taken by venipuncture of a vein draining the mid-section of the oviductal vasculature. Plasma was collected by centrifugation at 3,000 × g for 15 min and stored at -30°C until the progesterone assay was carried out. Progesterone was analyzed in duplicate using Coat-A-Count progesterone kits (Diagnostic Products, Los Angeles, CA), previously validated for use with porcine plasma without extraction (Chapter 3). Serial dilutions of pig plasma (100, 50, 25, 12.5 and 6.25 µl) showed parallelism to the standard curve. Assay sensitivity defined as 91.4% of total binding, was 0.009 ng/tube. The average intra- and inter-assay coefficients of variation were 10.4% and 10%, respectively.

Culture medium and luteal tissue progesterone RIA

Medium progesterone concentrations were determined using the same direct assay. The intra- and inter-assay coefficients of variation for these assays were 2.1% and 15.1%, respectively. Tissue progesterone RIA was based on the method described by Hunter et al. (1988). Aliquots (100 µl) of the ethanol extract were evaporated to dryness and redissolved in 1 ml assay buffer for direct assay of tissue progesterone content. Dilutions of extracted samples exhibited parallelism to the standard curve, and ethanol and buffer blanks were below the limit of sensitivity. The mean intra- and inter-coefficients of variation of these assays were 2.2% and 6.6%, respectively.

mRNA Expression Studies

For the purposes of mRNA expression studies for key steroidogenic enzymes as part of the current study, and for later studies on oviductal protein secretion, a sub-set of 10 animals from each treatment group were identified for detailed study on the basis of ovulation-to-surgery interval, peripheral plasma progesterone concentrations, and embryo developmental stage at surgery.

Total RNA Isolation

Total RNA was extracted from luteal tissue using TRIzol Reagent according to the manufacturer's instructions (Life Technologies, Gaithersburg, MD). The RNA was quantified by spectrophotometric absorbance at 260 nm, then aliquoted and stored at -80°C. RNA integrity was assessed by observing the 28S and 18S ribosomal RNA bands after electrophoresis of one aliquot on a 1% (w/v) formaldehyde agarose gel followed by ethidium bromide staining.

Northern Blot Analysis

Northern blots were prepared by subjecting 20 µg of total RNA to electrophoresis in 1% (w/v) agarose gels in duplicate under denaturing conditions with formaldehyde (Yuan et al., 1996), then transferred to Hybond-XL nylon membrane (Amersham Pharmacia Biotech, Baie d'Urfé, QC) overnight and baked at 80°C for 2 h under vacuum. Standard RNA size markers (Life Technologies, Gaithersburg, MD) were used to estimate the size of mRNA transcripts detected on Northern blots.

Blots were pre-hybridized for a minimum of 3 h at 60°C in 5 × SCC (single-strength SCC contains 150 mM sodium chloride, 15 mM citric acid), 100 µg/ml sheared salmon sperm DNA and 5 × Denhardt's solution (one hundred-strength Denhardt's contains 2% (w/v) bovine serum albumin, 2% (w/v) Ficoll, 2% (w/v) polyvinylpyrrolidone). Blots were then hybridized overnight in fresh prehybridization solution using porcine cDNA probes for StAR protein, P450scc (obtained from Drs. David Silversides and Bruce Murphy, CRRA, Université de Montreal, Canada) and 3beta-HSD (obtained from Dr. Fernand Labrie, CHUL Research Centre, Laval University, Canada) to assess steady state mRNA levels. The cDNA fragments were labeled with [^{32}P]-dATP (Amersham Pharmacia Biotech, Baie d'Urfé, QC) using a Random Primed DNA labeling kit according to manufacturer's instructions (Life Technologies, Gaithersburg, MD). Unincorporated nucleotides were removed using G-50 Sephadex Nick Columns (Amersham Pharmacia Biotech, Baie d'Urfé, QC). After hybridization, the blots were washed twice (15 min per wash) with 2 × saline sodium citrate (SSC)/0.1% (w/v) sodium dodecyl sulfate (SDS) at 35°C, followed by two washes (15 min per wash) with 1 × SSC/0.1% (w/v) SDS, and finally with one 15 min wash in 0.2 × SSC/0.1% (w/v) SDS at 60°C. Blots were exposed to BioMAX MS film (Eastman Kodak, Rochester, NY) for 96, 48 and 27 h for StAR, 3beta-HSD and P450scc, respectively, at -80°C with two intensifying screens. All RNA data were normalized for loading and are expressed as the following ratios: StAR/28S, 3beta-HSD/28S and P450scc/28S.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA expression was determined by semi-quantitative RT-PCR. Specific primers to amplify part of MMP-2, TIMP-1 and TIMP-2 mRNA were kindly provided by Dr. Denis Balcerzak (AFNS, University of Alberta, AB, Canada). The sequences of MMP-2 (510 bp) primers were 5'-CTT CCC CCG CCA GCC CAA GTG GG-3' (sense) and 5'-CGG GGG TAC TTC GGG ACA AGT GG-3' (antisense); TIMP-1 (504 bp) primers 5'-GTA CCT GCG TCC CAC CCC ACC-3' (sense) and 5'-GGC GGT GGA CCG GAC GGA CGG-3' (antisense); TIMP-2 (643 bp) primers 5'-CCT CCT GCT GCT GGG GAC GCT GC-3' (sense) and 5'-GGG

CAT TCG TCC GGT GGT CCT GA-3' (antisense). Primers for MMP-9 (357 bp) were synthesized according to Menino et al. (1997).

Before running the samples, the amount of total RNA, annealing temperature and cycle number of PCR were optimized empirically for each of the target genes. To verify that all the target genes were amplified in the linear range, one sample of RNA was serially diluted and each dilution was used to run all the reactions. The amount of total RNA selected was based on the sensitive, linear part of the dilution curve. Reverse transcription (RT) was carried out with M-MLV reverse transcriptase (64 U, Life Technologies, Gaithersburg, MD) at 37°C for 1 h in a total reaction volume of 5 ul, containing 1 μM primer, 0.25 mM each of dATP, dCTP, dGTP and dTTP, 50 mM Tris-HCL pH 8.3, 70 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol and 2.5 U RNase inhibitor (Life Technologies, Gaithersburg, MD). A blank was included with RT reactions, in which sterile water was substituted for RNA. PCR reactions were also performed without RT to confirm the absence of DNA contamination.

All PCR reactions were carried out in a Perkin Elmer GenAmp 2400 Thermocycler in a reaction volume of 50 ul, containing 2 U *Taq* polymerase (Life Technologies, Gaithersburg, MD), 0.6 μM primers, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 2 mM $MgCl_2$ and 0.2 mM dNTP mixture. For each gene, 30 samples were run at the same time and the PCR amplification of target genes for all samples was therefore assumed to be carried out under the same conditions. The identities of the amplified products were confirmed by diagnostic restriction enzyme analysis and DNA sequence analysis. RT-PCR amplification of sample RNA without adding reverse transcriptase did not yield products with the predicted sizes for any of the target genes. As a positive control Jag-1 porcine trophoblast cell line RNA (Ramsoondar et al., 1999) was run with the unknown sample for each gene. Aliquots of each PCR reaction (10 μl) were electrophoresed through a 2% (w/v) agarose gel stained with 0.1 μg/ml ethidium bromide. Gels were visualized on a UV transilluminator and photographed. The images were quantified using molecular analyst software (v6.0) (BIO-RAD, Mississauga, ON). The G3PDH gene (Yelich et al., 1997) was used as internal RT-PCR control to normalize the target gene expression. mRNA expression for all these genes is expressed as the ratio of target gene/G3PDH.

Zymography

Analysis of TIMP-1 mRNA expression revealed differences between HR+I, and RH and HR groups. To confirm this finding, gelatinase activity (MMP-2) was directly assessed by zymography (Driancourt et al., 1998) using available tissue. Total protein was extracted and quantified using the BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer's instructions. Fifteen μg of total protein was loaded on 17.5% (w/v) SDS-polyacrylamide resolving gels containing 1-mg gelatin/ml and electrophoresed under non-reducing conditions. At the completion of migration, the gels were washed once in 2.5% (v/v) Triton-×100 (Sigma, St. Louis, MO) for 30 min, twice in 50mM Tris-HCl pH 7.4 for 15 min, and then incubated for 20 h in enzyme buffer (50 mmol Tris-HCl/l, 10 mM $CaCl_2$, pH 7.4). Gelatinase activity was visualized as a clear band against a blue background after staining in Coomassie blue and destaining in water.

Statistical analysis

Progesterone content in luteal tissue before culture (T0 P4), and progesterone production and release by minced luteal tissue *in vitro* expressed as ng/mg tissue/3 h, were analyzed based on the data from 44 animals. The amount of progesterone secreted by dispersed luteal cells was expressed as ng/10^5 cells/3 h. Progesterone production was calculated by subtracting the progesterone content in tissue before incubation from the total progesterone in the medium (progesterone release) and in the tissue after incubation.

All dependent variables were analyzed for normality using the Wilk-Shapiro test (SAS, 1990). Data for the dependent variables of T0 P4, progesterone production and release by luteal tissue, and dispersed luteal cell progesterone production were analyzed using the general linear model (GLM) procedure of SAS (SAS, 1990). Since there was no difference in time interval from LH surge to ovulation among the three groups, progesterone content before culture was analyzed with littermate and treatment as main effects and with ovulation-to-surgery time interval (time) as a covariate. For progesterone release, progesterone production by tissue, luteal cell progesterone production, and responses to LH, sources of variation were littermate, treatment, LH, and LH × treatment interaction, with time as a covariate. If a time × treatment interaction was established, heterogenous slopes were used to analyze the effect of time within treatment. A multiple

comparison procedure was used to analyze LH effects within treatment (SAS, 1990). Analysis of the dependent variables of StAR protein, P450scc, 3beta-HSD, MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA expression was based on data from 10 animals per treatment as discussed earlier. Treatment effects were tested, fitting time as a covariate, by the GLM procedure of SAS (1990). Again, when a time × treatment interaction was detected, heterogeneous slopes were used for further analysis.

Linear regression analyses (SAS, 1990) were performed to determine the associations among progesterone release, progesterone production by tissue, and oviductal and peripheral plasma progesterone concentrations, and the associations between StAR protein, P450scc, 3beta-HSD mRNA expression, and progesterone production. Oviductal and peripheral plasma progesterone concentrations were logarithmically and square root transformed, respectively, to approach a normal distribution for regression analysis. All data are expressed as least squares means ± the standard error of the least squares means (LSMean ± SEM).

RESULTS

Progesterone production and release by luteal tissue, and by dispersed luteal cells

Overall (Table 5.1), treatment did not affect progesterone content in luteal tissue before culture (T0 P4) ($P>0.05$), but did affect progesterone production and release during *in vitro* culture by both minced tissue ($P<0.001$), and by dispersed luteal cells ($P<0.01$). Progesterone production and release by minced luteal tissue from HR+I gilts was higher than from HR and RH groups ($P<0.05$), with no difference between HR and RH groups ($P>0.05$). Progesterone production by dispersed luteal cells from HR+I gilts was also higher than that from HR gilts ($P<0.02$), with no difference between HR and RH, or between RH and HR+I gilts ($P>0.05$). A litter effect was established for progesterone production and release by minced luteal tissue *in vitro* ($P<0.001$).

Ovulation-to-surgery time interval (time) interacted with treatment ($P<0.01$) to affect progesterone production and release by luteal tissue (Figure 5.1A and B, respectively). Both progesterone production and release *in vitro* by luteal tissue from HR and RH gilts increased as ovulation-to-surgery time interval increased (progesterone production: $r = 0.84$ and 0.71; progesterone release: $r = 0.79$ and 0.82 for HR and RH

gilts, respectively; P<0.01). In contrast, progesterone production and release by luteal tissue from HR+I gilts were consistently high, regardless of when the tissue was collected (r = 0.28 and 0.1 for progesterone production and release, respectively; P>0.05).

Progesterone production and release from luteal tissue and cells in response to LH stimulation

Progesterone production by minced tissue from HR gilts did not show responses to LH stimulation, whereas progesterone production in luteal tissue from HR+I gilts was increased by stimulation with 1, 10 and 100 ng/ml LH and was enhanced at 10 ng/ml LH in the RH group, compared to the 0 ng/ml LH control (P<0.05; Figure 5.2A). Progesterone release by minced luteal tissue was increased at 10 and 100 ng/ml LH in HR+I and RH gilts (P<0.05), but again was not affected by LH in HR gilts (Figure 5.2B). Increasing doses of LH did not affect progesterone production by dispersed luteal cells (P>0.05; Figure 5.2C).

Correlation between progesterone production and release in vitro by luteal tissue and oviductal, and peripheral plasma progesterone concentrations

Oviductal and peripheral plasma progesterone concentrations were variable and were not normally distributed. However, after logarithmic and root square transformations of oviductal and plasma progesterone data, respectively, strong positive correlations were established between progesterone production *in vitro* by luteal tissue and oviductal (Figure 5.3A) and peripheral (Figure 5.3B) plasma progesterone concentrations, and between progesterone release by luteal tissue and oviductal (Figure 5.3C) and peripheral (Figure 5.3D) progesterone concentrations. Progesterone content in luteal tissue before culture was also associated with oviductal (r = 0.58, P<0.001) and peripheral (r = 0.64, P<0.001) plasma progesterone concentrations.

StAR protein, P450scc, and 3beta-HSD mRNA expression in luteal tissue

No differences in StAR protein, P450scc, and 3beta-HSD mRNA expression in luteal tissue were found among the three treatment groups (P>0.05; Figure 5.4). However, a time × treatment interaction was established (P<0.001). Similar to progesterone production and release results, mRNA expression for these three key

protein/enzymes increased in HR and RH groups as time interval increased ($P<0.01$), but was consistently high and not affected by time in HR+I gilts ($P>0.05$; Figure 5.5A, B, C). StAR protein, P450scc and 3beta-HSD mRNA expression in luteal tissue were positively correlated with de novo progesterone production by minced luteal tissue *in vitro* (Figure 5.6A, B, C).

MMP-2, MMP-9, TIMP-1 and TIMP-2 mRNA expression and MMP-2 activity in luteal tissue

Treatment did not affect MMP-2, MMP-9 and TIMP-2 mRNA expression in luteal tissue ($P>0.05$); However, treatment affected TIMP-1 mRNA expression ($P<0.01$) which was lower in luteal tissue from HR+I gilts than from HR and RH gilts (Figure 5.7). Time interval did not affect MMP-2, MMP-9, TIMP-1 or TIMP-2 mRNA expression ($P>0.05$). MMP-2 activity, as assessed by zymography and expressed as arbitrary units, was 2.7 ± 0.3 ($n=7$), 3.4 ± 0.3 ($n=7$), and 2.6 ± 0.6 ($n=2$) for HR, HR+I, and RH gilts, respectively ($P<0.08$).

DISCUSSION

Culture of porcine luteal cells in the presence of serum has been used to investigate the effects of hormonal treatments on steroidogenesis (Wiesak et al., 1994). However, serum contains a complex mixture of hormones, nutrients, and growth factors. Chemically defined, serum-free, culture systems have been used by Mondschein et al. (1989) and Picton et al. (1999) to study porcine granulosa cells *in vitro*, in order to avoid potential confounding effects of serum-derived factors, which might themselves be mediators of nutritional/metabolic effects on luteal function. We considered it appropriate to develop a similar serum-free system for short-term culture of luteal tissue recovered from the feed-restricted and insulin-treated gilts in this study. Furthermore, since co-cultures of large and small luteal cells produce more progesterone than either large or small luteal cells alone (Lemon and Mauleon, 1982), and since communication between large and small luteal cells, and between luteal cells and immune and endothelial cells, has been shown to be important for luteal function (Pate, 1996; Ferrara et al., 1998), we chose to compare the use of minced luteal tissue and dispersions of mixed small and large

luteal cells for assessment of luteal function *in vitro*. Although progesterone production and release by minced tissue were different between HR+I and RH groups, these differences were not apparent using cell culture. Furthermore, minced luteal tissue from RH and HR+I groups responded to LH, but dispersed luteal cells from all groups did not. This lack of sensitivity in dispersed cells may be related to the dispersion process itself, since Ascoli and Segaloff (1986) reported that after digestion with collagenase, LH receptors may be degraded and cannot be regenerated during short-term culture. Therefore, minced luteal tissue cultured in serum-free medium *in vitro* appears to provide the most physiologically meaningful assessment of luteal function.

The results of the present study provide convincing evidence for the dynamic changes which occur in porcine luteal function in the immediate post-ovulatory period. Consistent with the more limited data of Ricke et al. (1999) on progesterone content and 3beta-HSD protein expression, we observed a rapid increase in progesterone production, progesterone release and mRNA expression for StAR protein, P450scc, and 3beta-HSD in HR and RH gilts in the 12- to 24-h period after ovulation. Our observation that progesterone content in luteal tissue was highly correlated with both oviductal and peripheral plasma progesterone concentrations further extends the data of Ricke et al. (1999), and suggest that because of the activity of the sub-ovarian countercurrent transfer system in pigs (Krzymowski et al., 1990), oviductal progesterone concentrations will reflect changes in early luteal function and exert regulatory effects on the oviduct (Murray et al., 1995). Although Ricke et al. (1999) indicated that luteal progesterone content provides a good index of normal luteal function, our data suggest that progesterone production and release by luteal tissue *in vitro* are better measures of luteal status. For example, progesterone production and release in tissue recovered from the HR+I group was higher than in the HR and RH groups, whereas luteal tissue progesterone content did not differ among the three treatments. Evidence for litter effects on progesterone production and release *in vitro* indicates the advantage of using littermates as part of well-controlled experiments to study nutritional effects on reproductive function in litter bearing mammals.

The present study showed that both progesterone production and release by luteal tissue, and mRNA expression for three key steroidogenic enzymes, did not differ between

HR and RH groups. However, when stimulated with physiological doses of LH, progesterone production and release by luteal tissue from RH, but not from HR gilts, increased. This is the first report to show that previous feed restriction (in this case during the second week of the estrous cycle) affects subsequent luteal function in the immediate post-ovulatory period by decreasing sensitivity to LH. This may be one cause for lower circulating progesterone concentrations and for the delayed rise in plasma progesterone concentrations in HR gilts in the immediate post-ovulatory period reported by Almeida et al. (2000a,b). According to the studies on swine follicular growth performed by Morbeck et al. (1992), it takes about 14 days for follicles to grow from antrum formation to 3 mm in diameter, with an additional 5 days of growth to reach preovulatory status. As the group of preovulatory follicles are recruited from a pool of 3 to 5 mm sized follicles bewteen d 14 and 16 of the estrous cycle (Foxcroft and Hunter 1985), feed restriction during the second week of the cycle would be coincident with the emergence of follicles into the recruitable pool. This could affect follicular development by a number of mechanisms, including central effects on gonadotropin secretion (Booth et al., 1994) and local effects on ovarian function (Cosgrove et al., 1992; Cosgrove and Foxcroft, 1996), and thus subsequently affect luteal function after ovulation. Evidence for such treatment effects on follicular maturation and endocrine status in the peri-ovulatory period in the same population of gilts used for the present study of luteal function, have recently been obtained by Almeida et al. (2000b). These data show that peak follicular phase estradiol concentrations and the size of the preovulatory LH surge were higher in RH than in HR gilts. Less et al. (1998) demonstrated that bovine granulosa cells undergoing luteinization *in vitro* produced more progesterone following exposure to a high LH 'surge' (15-100 ng/ml) than when they were exposed to lower doses of LH (0-10 ng/ml). *In vivo*, progesterone concentrations during the cycle were lower in cattle induced to ovulate with a low dose (2 μg) of a long-acting GnRH agonist (Buserelin) compared to the cattle administered higher doses of Buserelin (2 × 8 μg) or hCG (5000 IU). Thus, the higher LH surge in RH gilts, associated with a greater sensitivity to LH stimulation, would both contribute to more rapid luteinization of granulosa cells, and the quicker rise in peripheral progesterone concentrations reported by Almeida et al. (2000b).

The current experiment also demonstrated that exogenous insulin treatment during d 8 to 15 of the estrous cycle also affected subsequent luteal function, as evidenced by increased progesterone production and release by luteal tissue *in vitro*, enhanced StAR protein, P450scc, and 3beta-HSD mRNA expression, and decreased TIMP-1 mRNA expression in luteal tissue from HR+I gilts. Several mechanisms might mediate such insulin effects. *In vivo* studies have demonstrated that exogenous insulin treatment for 4 days in gonadotropin-treated prepubertal gilts increased the number of follicles ≤3 mm (Matamoros et al., 1991). Exogenous insulin also maintained the 4-6 mm size follicle population in cyclic gilts when insulin treatment began on d 15 of the cycle (Matamoros et al., 1990). In primiparous sows, insulin treatment for 3 days after weaning also increased the number of large follicles in these sows (Whiteley et al., 1998a,b). Insulin appears to act by reducing follicular atresia (Meurer et al., 1991a,b; Matamoros et al., 1990, 1991) and by suppressing follicular apoptosis (Purvis et al., 1997). Conversely, withdrawal of insulin in Streptozotocin-induced diabetic pigs reduced follicle diameter and estradiol production, and increased atresia (Cox et al., 1994; Edwards et al., 1996). Taken together, these data suggest that insulin treatment from d 8 to 15 of the cycle most likely enhances follicular development, and this was reflected by higher peak estradiol concentrations and an increase in the magnitude of the preovulatory LH surge in HR+I compared to HR gilts, and also in an increase in ovulation rate (Almeida et al., 2000b).

At the cellular level, porcine granulosa cells possess insulin receptors (Rein and Schomberg, 1982). Insulin can both interact with its own receptor and cross-react with IGF-I receptors to regulate folliculogenesis (McArdle et al., 1991). However, several lines of evidence *in vitro* suggest that insulin receptors mediate the actions of insulin. Studies in human granulosa cells showed that insulin-stimulated steroid production could be inhibited by anti-insulin receptor antibodies, but not by antibodies against the IGF-I receptor (Willis and Frank., 1995). Again, Nestler et al. (1998) demonstrated that insulin stimulation of testosterone production could not be inhibited by an antibody against IGF-I receptor in cultured human thecal cells, suggesting that this effect of insulin was also mediated by the insulin receptor. In i*n vivo* studies in rats, insulin treatment up-regulated IGF-I receptors (Poretsky et al., 1988) and may exert indirect actions by increasing sensitivity to IGF-I. The important action of insulin is to act synergistically with FSH in

granulosa cells, and with LH in theca cells, to enhance follicular development (Poretsky et al., 1999). *In vitro* culture of porcine granulosa cells from 1 to 3 mm follicles demonstrated that combined insulin and FSH treatment increased the formation of gap junction and microvilli, and enhanced the development of the smooth endoplasmic reticulum and the Golgi complex, relative to treatment with either hormone alone. The combined treatment also produced larger mitochondria with tubular cristae (Amsterdam et al., 1988). During the estrous cycle, the concentration of LH receptors on porcine luteal cells was lower immediately after ovulation, compared to the number of receptors either on preovulatory granulosa cells, or on mid-cycle (d 10) luteal cells (Gebarowska et al., 1997). In addition, the affinity of LH receptors on luteal cells was also lower compared with granulosa cells. Therefore, it is possible that the low number and affinity of LH receptors on developing luteal cells could be the mechanism responsible for the low sensitivity of porcine luteal tissue to LH stimulation as seen in HR gilts in the current study, where the luteal tissue was collected 12-24 h after ovulation. However, as combined insulin and FSH treatment acted synergistically to increase the number of LH receptors on porcine granulosa cells *in vitro* (Amsterdam et al., 1988), this would explain the high sensitivity of luteal tissue from HR+I gilts to LH stimulation.

Luteal progesterone production *in vitro* was higher in the HR+I than in the HR and RH groups, whereas expression of mRNA for StAR protein, P450scc, 3beta-HSD in luteal tissue from HR+I gilts did not differ from that in HR and RH gilts. This discrepancy may be also explained by the difference in sensitivity to LH receptors. Yuan et al. (1996) demonstrated that administration of recombinant porcine somatotropin decreased luteal function by affecting LH receptor gene expression, but did not affect the mRNA expression for 3beta-HSD or P450scc. However, from a functional perspective, there was a close association in the present study between progesterone production and mRNA expression for three of the steroidogenic enzymes over the different ovulation-to-surgery time intervals seen in HR, HR+I and RH gilts. Progesterone production and mRNA expression for three of the steroidogenic enzymes was consistently high in HR+I gilts regardless of the ovulation-to-surgery time interval, whereas in the RH and HR gilts, progesterone production and mRNA expression both increased as this time interval increased. Besides, progesterone production by luteal tissue *in vitro* was strongly

correlated with circulating plasma progesterone concentrations. We conclude that insulin treatment increased progesterone production by accelerating the temporal up-regulation of StAR protein, P450scc, 3beta-HSD mRNA expression during the luteinization process. This would be another mechanism contributing to the quicker rise in circulating progesterone concentrations in HR+I gilts reported by Almeida et al. (2000b). The close associations between gene expression, luteal function *in vitro*, and peripheral plasma progesterone concentrations, suggest that treatment effects on gene expression would also be a mechanism mediating the difference in the rate of increase in plasma progesterone between HR and RH gilts in the data of Almeida et al. (2000b). The lack of such differences is probably explained by the relatively small sub-sample of gilts used for the gene expression studies reported here.

The primary regulator of luteal function is luteinizing hormone (LH), which regulates progesterone synthesis and release via classic endocrine mechanisms. However, in addition to this systemic mechanism, intraovarian regulatory systems are involved in modulating the response of corpora lutea to circulating gonadotropin (Adashi, 1994; deMoura et al., 1997). IGF-I (Urban et al., 1990; Caubo et al., 1989), FGF (Biswas et al, 1988), and TGFβ (Kubota et al., 1994) affect gonadotrophin-induced granulosa cell progesterone secretion *in vitro* in pigs. In order to be active, these growth factors require proteolysis of their binding protein (IGF-I), proteolytic release from the extracellular matrix (FGF), or proteolytic activation from a latent form (TGFβ). To our knowledge, our data are the first to demonstrate that previous insulin treatment produces latent effects on TIMP-1 mRNA expression and possibly on MMP-2 activity in luteal tissue. An increase in MMP-2 activity could, in turn, increase availability of IGF-I following IGF binding protein proteolysis (Fowlkes et al., 1994). The resulting increase in free IGF-I would then more efficiently stimulate progesterone production in HR+I gilts (Urban et al., 1990). Very similar mechanisms have also been reported by Driancourt et al. (1998) as possible mediators of enhanced progesterone production during luteinization in the high prolific Chinese Meishan pigs.

In conclusion, *in vitro* culture of minced luteal tissue in serum-free medium was a suitable system in which to study luteal function in previously feed-restricted and insulin-treated animals. Feed restriction during the second week of the estrous cycle appears to

decrease circulating progesterone concentrations by affecting the sensitivity of the luteal tissue to LH stimulation. Concomitant insulin treatment during this period of feed restriction had lasting, beneficial effects on luteal function, increasing progesterone production and release by increasing the sensitivity to LH stimulation, by affecting steroidogenic enzyme gene expression, and possibly by increasing the availability of IGF-I in the corpus luteum through downstream effects on the MMP/TIMP cascade. Overall, our data suggest that gene expression for key steroidogenic enzymes is tightly linked to temporal changes in progesterone production and release in the early luteal phase in the pig.

LITERATURE CITED

Adashi EY, Rohan RM. Intraovarian regulation: Peptidergic signalling system. Trends Endocrinol Metab 1992; 3: 243-248.

Adashi EY. Growth factors and ovarian function: the IGF-I paradigm. Horm Res 1994; 42: 44-48.

Almeida FRCL, Kirkwood RN, Aherne FX, Foxcroft GR. Consequences of different patterns of feed intake during the estrous cycle in gilts on subsequent fertility. J Anim Sci 2000a; 78: 1556-1563.

Almeida FRCL, Mao J, Novak S, Cosgrove JR, Foxcroft GR. Effects of patterns of feed restriction and insulin treatment during the luteal phase on reproductive, metabolic and endocrine parameters in cyclic gilts. 2000b; (submitted to JAS)

Amsterdam A, May JV, Schomberg DW. Synergistic effect of insulin and follicle-stimulating hormone on biochemical and morphological differentiation porcine granulosa cells *in vitro*. Biol Reprod 1988; 39: 379-390.

Ascoli M, Segaloff DL. Effects of collagenase on the structure of the lutropin/ choriogonadotropin receptor. J Biol Chem 1986; 261: 3807-3815.

Ashworth, CJ. Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. Anim Reprod Sci 1991; 26: 311-321

Beltranena E, Aherne FX, Foxcroft GR, Kirkwood RN. Effects of pre- and postpubertal feeding on production traits at first and second estrus in gilts. J Anim Sci 1991; 69: 886-893.

Biswas SB, Hammond RW, Anderson LD. Fibroblast growth factors from bovine pituitary and human placenta and their functions in the maturation of porcine granulosa cells *in vitro*. Endocrinology 1988; 123: 559-566.

Booth PJ, Craigon J, Foxcroft GR. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J Anim Sci 1994; 72: 2415-2424.

Britt JH, Armstrong JD, Cox NM. Metabolic interfaces between nutrition and reproduction in pigs. Proc 11th Int Cong Anim Reprod AI 1988; 5: 117-126.

Carr BR. The ovary. In: Carr BR, Blackwell RE (eds.), Textbook of Reproductive Medicine (2nd ed.) Appleton & Lange, Stamford, Connecticut; 1998; 207-231.

Caubo B, DeVinna RS, Tonetta SA. Regulation of steroidogenesis in cultured porcine theca cells by growth factors. Endocrinology 1989; 125: 321-326.

Cosgrove JR, Foxcroft GR. Nutrition and reproduction in the pig: ovarian aetiology. Anim Reprod Sci 1996; 42: 131-141.

Cosgrove JR, Tilton JE, Hunter MG, Foxcroft GR. Gonadotropin-independent mechanisms participate in ovarian responses to realimentation in feed-restricted prepubertal gilts. Biol Reprod 1992; 47: 736-745.

Cox NM, Meurer KA, Carlton CA, Tubbs RC, Mannis DP. Effect of diabetes mellitus during the luteal phase of the oestrous cycle on preovulatory follicular function, ovulation and gonadotrophins in gilts. J Reprod Fertil 1994; 101: 77-86.

Cox NM, Stuart MJ, Althen TG, Bennett WA, Miller HW. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J Anim Sci 1987; 64: 507-516.

deMoura MD, Choi D, Adashi EY, Payne DW. Insulin-like growth factor-I-mediated amplification of follicle-stimulating hormone-supported progesterone accumulation by cultured rat granulosa cells: enhancement of steroidogenic enzyme activity and expression. Biol Reprod 1997; 56: 946-953.

Driancourt MA, Quesnel H, Meduri G, Prunier A, Hermier D. Luteinization and proteolysis in ovarian follicles of Meishan and Large White gilts during the preovulatory period. J Reprod Fertil 1998; 114: 287-297.

Edwards JL, Hughey TC, Moore AB, Cox NM. Depletion of insulin in streptozocin-induced-diabetic pigs alters estradiol, insulin-like growth factor (IGF)-I and IGF binding proteins in cultured ovarian follicles. Biol Reprod 1996; 55: 775-781.

Ferrara N, Chen H, Smyth TD, Gerber HP, Nguyen TN, Peers D, Chisholm V, Hillan KJ, Schwall RH. Vascular endothelial growth factor is essential for corpus luteum angiogenesis. Nat Med 1998; 4: 336-340.

Flowers B, Martin MJ,, Cantley TC, Day BN. Endocrine changes associated with a dietary-induced increase in ovulation rate (flushing) in gilts. J Anim Sci 1989; 67: 771-778.

Fowlkes JL, Suzuki K, Nagase H, Thrailkill KM. Proteolysis of insulin-like growth factor binding protein-3 during rat pregnancy: a role for matrix metalloproteinases. Endocrinology 1994; 135: 2810-2813.

Foxcroft GR, Hunter MG. Basic physiology of follicular maturation in the pig. J Reprod Fertil Suppl 1985; 33: 1-19.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Gadsby JE, Lovdal JA, Samaras S, Barber JS, Hammond JM. Expression of the messenger ribonucleic acids for insulin-like growth factor-I and insulin-like growth factor binding proteins in porcine corpora lutea. Biol Reprod 1996; 54: 339-346.

Gebarowska D, Ziecik AJ, Gregoraszczuk EL. Luteinizing hormone receptors on granulosa cells from preovulatory follicles and luteal cells throughout the oestrous cycle of pigs. Anim Reprod Sci 1997; 49: 191-205.

Hunter MG, Picton HM, Biggs C, Mann GE, McNeilly AS, Foxcroft GR. Periovulatory endocrinology in high ovulating Meishan sows. J Endocrinol 1996; 150: 141-147.

Hunter MG, Southee JA, Lamming GE. Function of abnormal corpora lutea *in vitro* after GnRH-induced ovulation in the anoestrous ewe. J Reprod Fertil 1988; 84: 139-148.

Hunter MG. Responsiveness *in vitro* of porcine luteal tissue recovered at two stages of the luteal phase. J Reprod Fertil 1981; 63: 471-476.

Jindal R, Cosgrove JR, Foxcroft GR. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. J Anim Sci 1997; 75: 1063-1070.

Krzymowski T, Kotwica J, Krzymowska SS. Uterine and ovarian countercurrent pathways in the control of ovarian function in the pig. J Reprod Fertil Suppl 1990; 40: 179-191.

Kubota T, Kamada S, Taguchi M, Aso T. Autocrine/paracrine function of transforming growth factor-beta 1 in porcine granulosa cells. Hum Reprod 1994; 9: 2118-2122.

Lemon M, Mauleon P. Interaction between two luteal cell types from the corpus luteum of the sow in progesterone synthesis *in vitro*. J Reprod Fertil 1982; 64: 315-323.

Less M, Wolfenson D, Shaham A, Roth Z, Meidan R. Effects of LH surge pattern on progesterone secretion during development of the corpus luteum and *in vitro* luteinization of granulosa cells in cows. J Reprod Fertil Abstr Ser 1998; 21: 33 Abst 76.

Matamoros IA, Cox NM, Moore AB. Exogenous insulin and additional energy affect follicular distribution, follicular steroid concentrations, and granulosa cell human chorionic gonadotropin binding in swine. Biol Reprod 1990; 43: 1-7.

Matamoros IA, Cox NM, Moore AB. Effects of exogenous insulin and body condition on metabolic hormones and gonadotropin-induced follicular development in prepuberal gilts. J Anim Sci 1991; 69: 2081-2091.

McArdle CA, Kohl C, Rieger K, Groner I, Wehrenberg U. Effects of gonadotropins, insulin and insulin-like growth factor I on ovarian oxytocin and progesterone production. Mol Cell Endocrinol 1991; 78: 211-220.

Menino AR Jr, Hogan A, Schultz GA, Novak S, Dixon W, Foxcroft GH. Expression of proteinases and proteinase inhibitors during embryo-uterine contact in the pig. Dev Gen 1997; 21: 68-74.

Meurer KA, Cox NM, Matamoros IA, Tubbs RC. Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J Reprod Fertil 1991a; 91: 187-196.

Meurer KA, Cox NM, Tubbs RC. Absence of insulin affects recruitment and maintenance of preovulatory follicles in cyclic diabetic gilts. J Anim Sci 1991b; 68 (Suppl. 1): 466.

Mondschein JS, Canning SF, Miller DQ, Hammond JM. Insulin-like growth factors (IGFs) as autocrine/paracrine regulators of granulosa cell differentiation and growth: studies with a neutralizing monoclonal antibody to IGF-I. Biol Reprod 1989; 41: 79-85.

Morbeck D, Esbenshade KL, Flowers WL, Britt JH . Kinetics of follicle growth in the prepubertal gilt. Biol Reprod 1992; 47: 485-491.

Murray MK, DeSouza MM, Messinger SM. Oviduct during early pregnancy: hormonal regulation and interactions with the fertilized ovum. Microsc Res Tech 1995; 31: 497-506.

Nestler JE, Jakubowicz DJ, de Vargas AF, Brik C, Quintero N, Medina F. Insulin stimulates testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 1998; 83: 2001-2005.

Orly J, Stocco DM. The role of the steroidogenic acute regulatory (StAR) protein in female reproductive tissues. Horm Met Res 1999; 31: 389-398.

Pate JL. Intercellular communication in the bovine corpus luteum. Theriogenology 1996; 45: 1381-1397.

Picton HM, Campbell BK, Hunter MG. Maintenance of oestradiol production and expression of cytochrome P450 aromatase enzyme mRNA in long-term serum-free cultures of pig granulosa cells. J Reprod Fertil 1999; 115: 67-77.

Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC. The insulin-related ovarian regulatory system in health and disease. Endocr Rev 1999; 20: 535-582.

Poretsky L, Glover B, Laumas V, Kalin M, Dunaif A. The effects of experimental hyperinsulinemia on steroid secretion, ovarian [^{125}I]insulin binding, and ovarian [^{125}I]insulin-like growth-factor I binding in the rat. Endocrinology 1988; 122: 581-585.

Purvis J, Skelton JO, Quirk MN, Moore AB, Whitley NC, Cox NM. Influence of insulin and insulin-like growth factor-I (IGF-I) on the function of porcine ovarian follicles in culture. J Anim Sci 1997; 75(Suppl. 1): 1.

Ramirez JL, NM Cox, Moore AB. Influence of exogenous insulin before breeding on conception rate and litter size of sows. J Anim Sci 1997; 75: 1893-1898.

Ramsoondar JJ, Christopherson RJ, Guilbert LJ, Dixon WT, Ghahary A, Ellis S, Wegmann TG, Piedrahita JA. Lack of class I major histocompatibility antigens on trophoblast of periimplantation blastocysts and term placenta in the pig. Biol Reprod 1999; 60: 387-397.

Rein MS, Schomberg DW. Characterization of insulin receptors on porcine granulosa cells. Biol Reprod 1982; 26 (Suppl. 1): 113.

Ricke WA, Redmer DA, Reynolds LP. Growth and cellular proliferation of pig corpora lutea throughout the oestrous cycle. J Reprod Fertil 1999; 117: 369-377.

SAS. SAS/STAT User's Guide (Version 6.06 Ed.). Cary, NC: Statistical Analysis System Institute Inc. 1990.

Smith MF, McIntush EW, Smith GW. Mechanisms associated with corpus luteum development. J Anim Sci 1994; 72: 1857-1872.

Urban RJ, Garmey JC, Shupnik MA, Veldhuis JD. Insulin-like growth factor type I increases concentrations of messenger ribonucleic acid encoding cytochrome P450 cholesterol side-chain cleavage enzyme in primary cultures of porcine granulosa cells. Endocrinology 1990; 127: 2481-2488.

van den Brand H, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: II. Effects on periestrus hormone profiles and embryonal survival. J Anim Sci 2000; 78: 405-411.

Whitley NC, Moore AB, Cox NM. Comparative effects of insulin and porcine somatotropin on postweaning follicular development in primiparous sows. J Anim Sci 1998a; 76: 1455-1462.

Whitley NC, Thomas MNQ, Skelton JO, Moore AB, Purvis J, Qiu Y, Cox NM. Influence of insulin on follicular development and the intrafollicular insulin-like growth factor I (IGF-I) system in sows after weaning. J Reprod Fertil 1998b; 112: 175-184.

Wiesak T, Hardin RT, Foxcroft GR. Evaluation of *in vitro* culture conditions to demonstrate pregnancy-dependent changes in luteal function in the pig. Biol Reprod 1994; 51: 254-261.

Willis D, Franks S. Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. J Clin Endocrinol Metab 1995; 80: 3788-3790.

Yelich JV, Pomp D, Geisert RD. Ontogeny of elongation and gene expression in the early developing porcine conceptus. Biol Reprod 1997; 57: 1256-1265.

Yuan W, Sterle JA, Cantley TC, Lamberson WR, Day BN, MC Lucy. Responses of porcine corpora lutea to somatotropin administration during pregnancy. J Anim Sci 1996; 74: 873-878.

Table 5.1. LSMeans (± SEM) of progesterone (P4) content in pooled luteal tissue (CL) before (time zero progesterone, T0 P4) and after culture, and P4 production and release during culture by luteal tissue, and P4 production by dispersed luteal cells, recovered from HR, HR+I, and RH gilts (see text for full description).

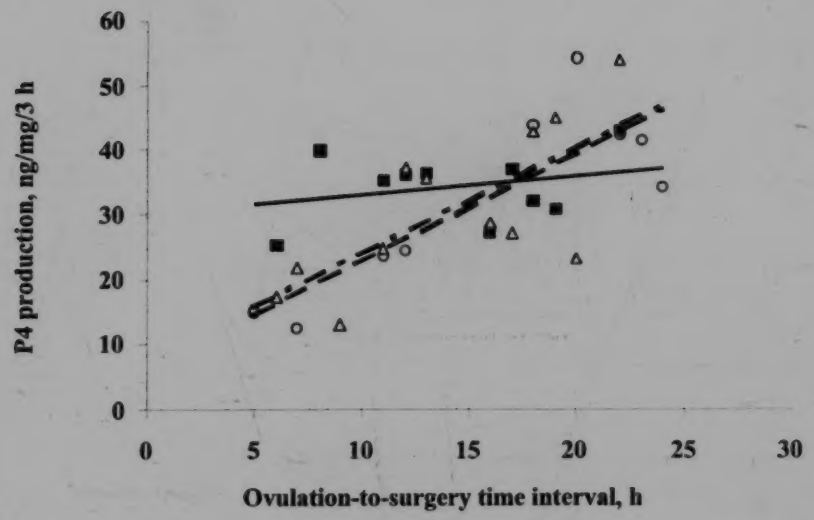
	Treatment		
Item	HR	HR+I	RH
N	15	15	14
T0 P4 content in CL, ng/mg	10.6 ± 1.2	13.5 ± 1.9	11.8 ± 1.5
Minced luteal tissue:			
P4 content in CL after culture, ng/mg	26.1 ± 0.8	28.0 ± 0.8	26.6 ± 0.8
P4 production, ng/mg/3 h	32.4 ± 1.0^{a}	35.2 ± 1.0^{b}	32.3 ±1.1^{a}
P4 release into medium, ng/mg/3 h	16.9 ± 0.6^{x}	20.7 ± 0.6^{y}	17.8 ± 0.7^{x}
Luteal cells:			
P4 production, ng/100,000 cells/3 h	9.9 ± 0.7^{a}	12.2 ± 0.6^{b}	10.7 ± 0.7$^{a, b}$

$^{a, b}$: Means within a row lacking common superscripts differ (P=0.05);

$^{x, y}$: Means within a row lacking common superscripts differ (P<0.01)

Figure 5.1. Correlation between progesterone (P4) production (A) and release (B) by minced luteal tissue *in vitro* (ng/mg/3 h) and ovulation-to-surgery time interval in HR (○, - - -), HR+I (■, ——) and RH (△,-·-) gilts. Since the plots of progesterone data over time were similar at five different LH levels, data plotted in this figure were based on pooled means for each time point.

(A)


P4 production, ng/mg/3 h
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Ovulation-to-surgery time interval, h

(B)

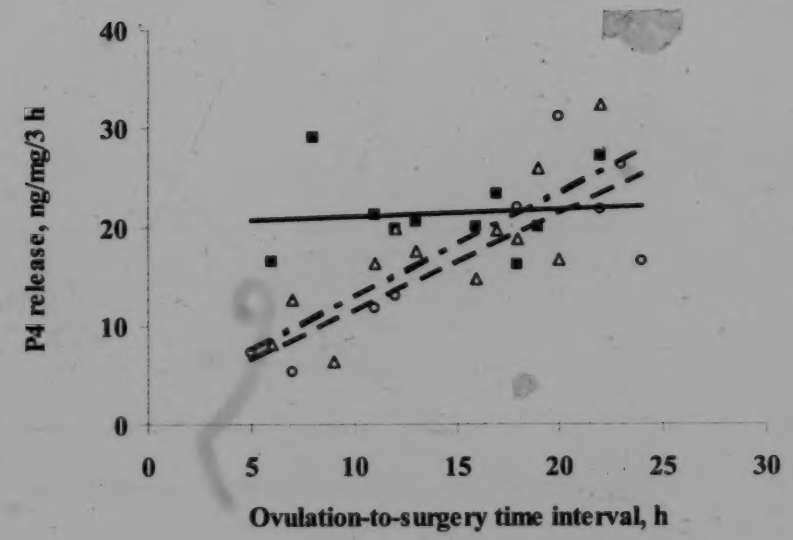

P4 release, ng/mg/3 h
40
30
20
10
0
0
5
10
15
20
25
30
Ovulation-to-surgery time interval, h

Figure 5.2. Progesterone (P4) production (A) and release (B) *in vitro* by luteal tissue from HR, HR+I, and RH gilts, and progesterone production by dispersed luteal cells (C; pooled data) in response to LH stimulation. The bars represent LS-means ± SEM. *: Significant difference within treatment ($P<0.05$) to the 0 ng/ml control.

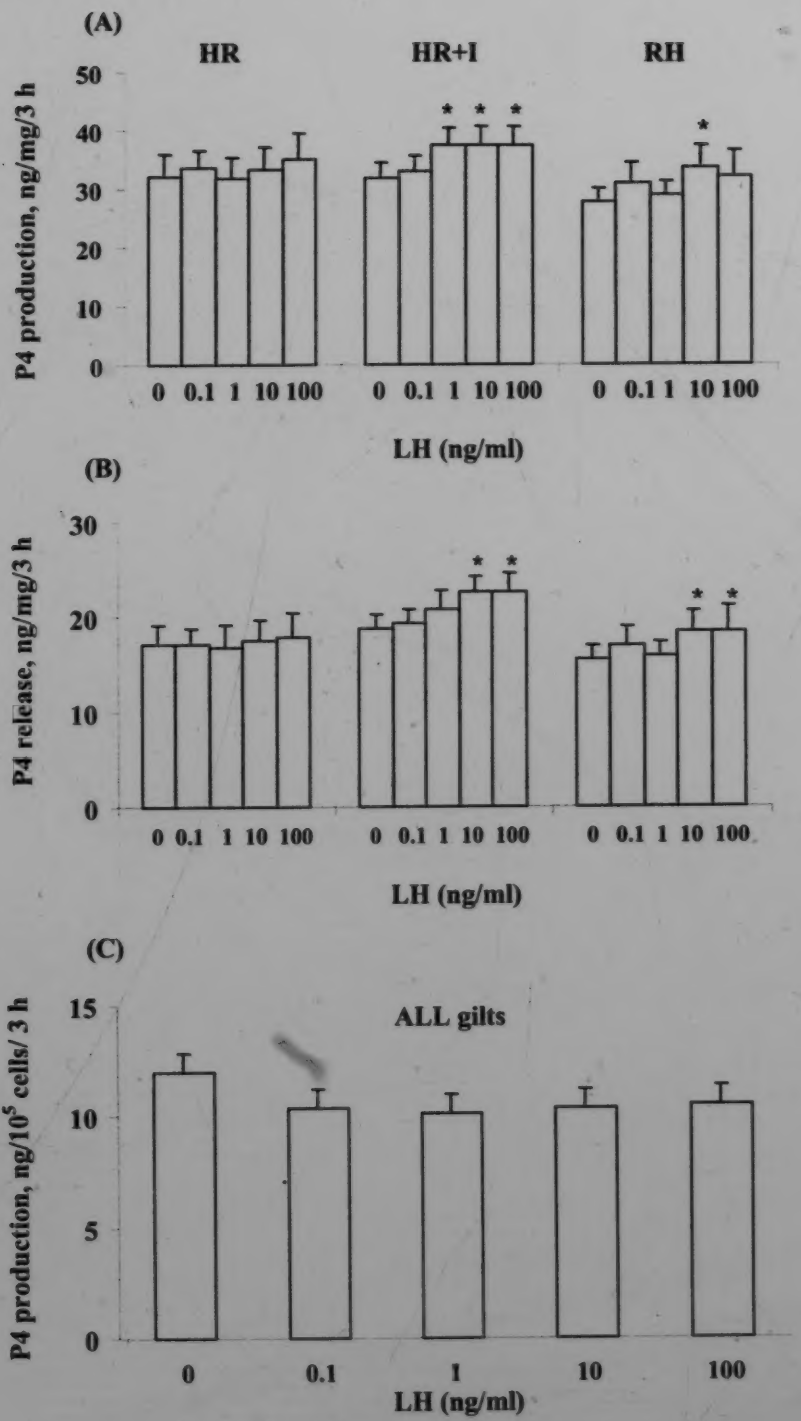
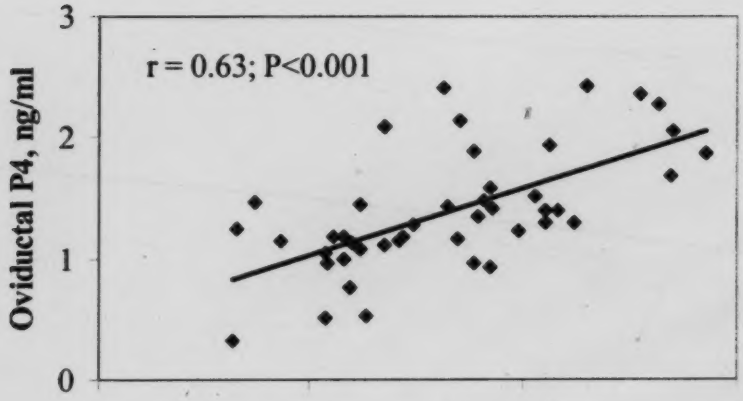
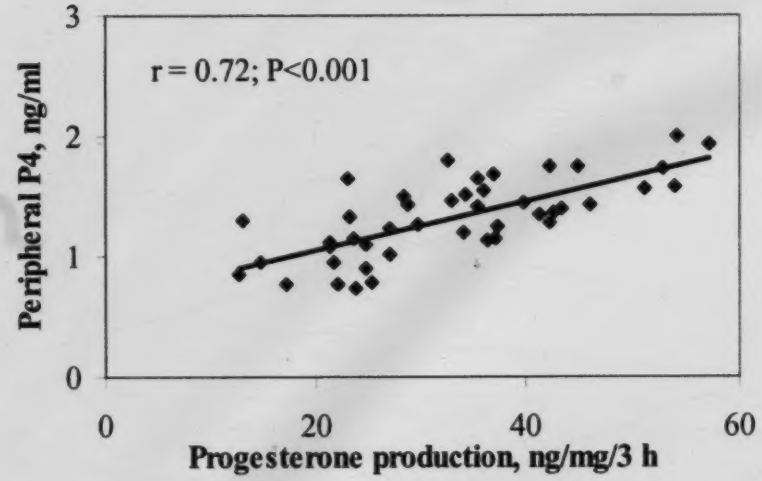

(A)
HR
HR+I
RH
P4 production, ng/mg/3 h
50
40
30
20
10
0
0 0.1 1 10 100
LH (ng/ml)
(B)
P4 release, ng/mg/3 h
30
20
10
0
0 0.1 1 10 100
LH (ng/ml)
(C)
ALL gilts
P4 production, ng/10^5 cells/ 3 h
15
10
5
0
0 0.1 1 10 100
LH (ng/ml)

Figure 5.3. Correlation between progesterone production by minced luteal tissue *in vitro* and oviductal (A), and peripheral (B) progesterone (P4) concentrations at the time of surgery, and between progesterone release by luteal tissue *in vitro* and oviductal (C), and peripheral (D) progesterone concentrations at the time of surgery (n=44). Oviductal and peripheral progesterone data were logarithm and, root square transformed, respectively. The distribution was similar among HR, HR+I, and RH groups.

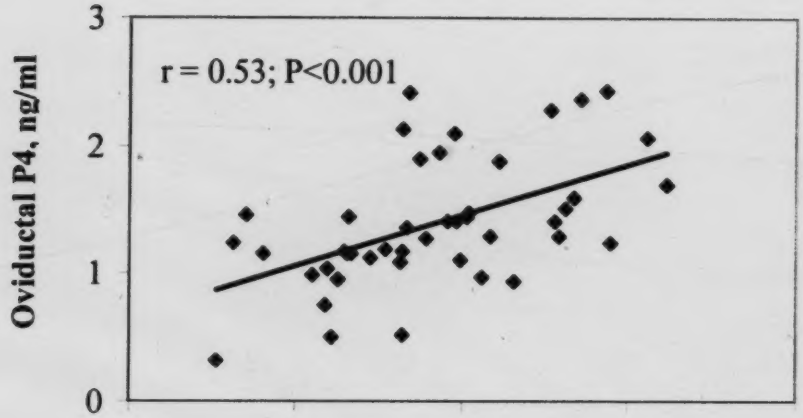
(A)


r = 0.63; P<0.001
Oviductal P4, ng/ml
0
1
2
3

(B)


r = 0.72; P<0.001
Peripheral P4, ng/ml
0
1
2
3
0
20
40
60
Progesterone production, ng/mg/3 h

(C)


r = 0.53; P<0.001
Oviductal P4, ng/ml
0
1
2
3

(D)

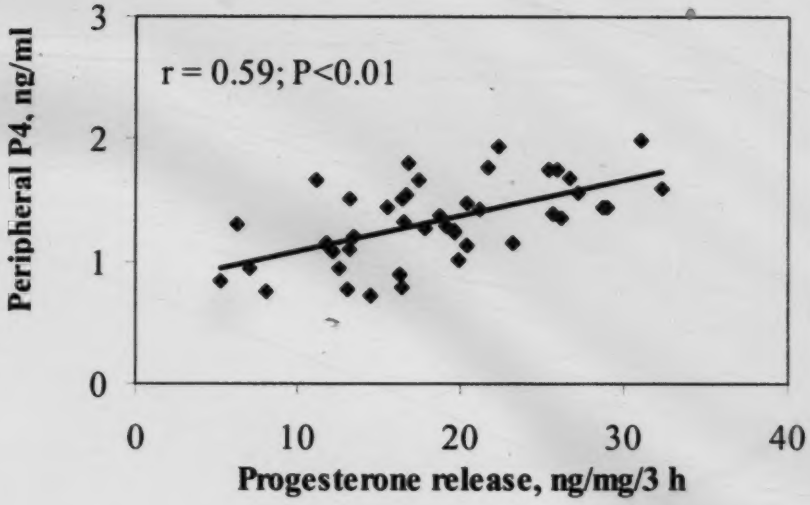

r = 0.59; P<0.01
Peripheral P4, ng/ml
0
1
2
3
0
10
20
30
40
Progesterone release, ng/mg/3 h

Figure 5.4. StAR, P450scc, and 3beta-HSD mRNA expression in luteal tissue from HR, HR+I and RH groups. mRNA expression was normalized for loading and expressed as arbitrary units. Bars represent LSMeans ± SEM.

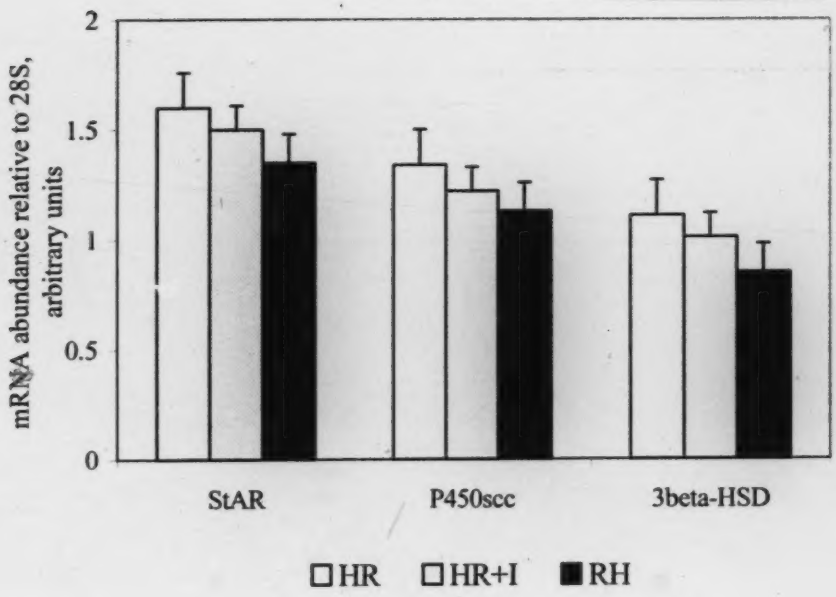
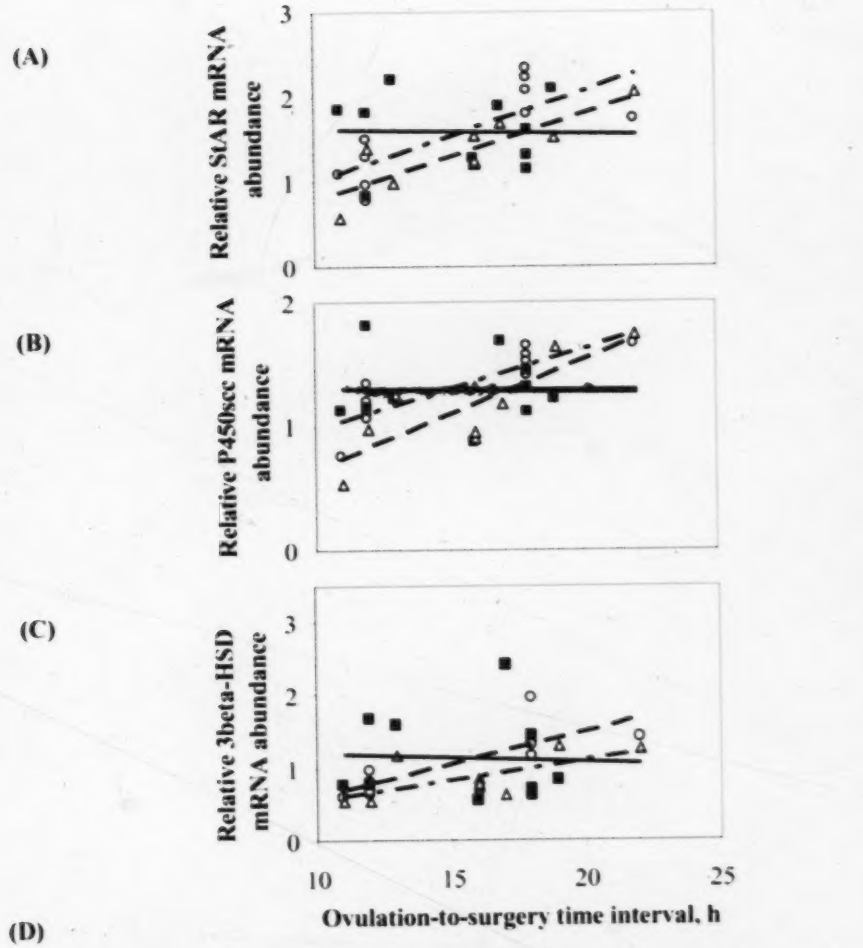

mRNA abundance relative to 28S, arbitrary units
2
1.5
1
0.5
0
StAR
P450scc
3beta-HSD
HR
HR+I
RH

Figure 5.5. StAR protein (A), P450scc (B), and 3beta-HSD (C) mRNA expression in luteal tissue relative to ovulation-to-surgery time interval in HR (O, - - -), HR+I (■, ——) and RH (△, -·-) gilts. As the interval increases, mRNA expression for these three key enzyme increased in HR and RH gilts ($P<0.01$), but was consistently high in HR+I pigs ($P>0.05$). The bottom panels (D) show representative P450scc transcript expression and loaded total RNA from one gel. Samples were organized in time order from shortest to longest interval from right tc left within each treatment.


(A)
Relative StAR mRNA abundance
3
2
1
0
(B)
Relative P450scc mRNA abundance
2
1
0
(C)
Relative 3beta-HSD mRNA abundance
3
2
1
0
10
15
20
25
Ovulation-to-surgery time interval, h

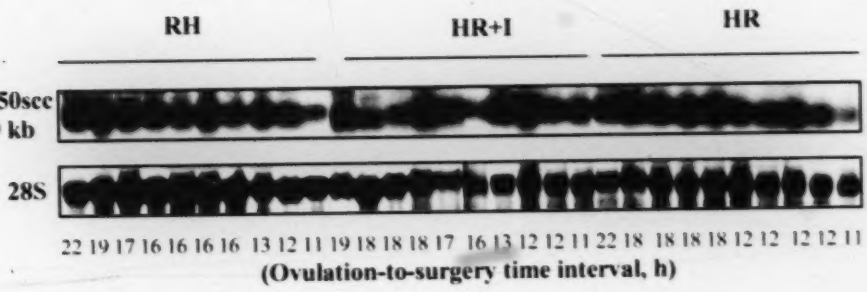

(D)
RH
HR+I
HR
P450scc 1.9 kb
28S
22 19 17 16 16 16 16 13 12 11 19 18 18 18 17 16 13 12 12 11 22 18 18 18 18 12 12 12 12 11
(Ovulation-to-surgery time interval, h)

Figure 5.6. Correlation between StAR protein (A), P450scc (B), and 3beta-HSD (C) mRNA expression in luteal tissue and progesterone production by luteal tissue *in vitro* (n=29).

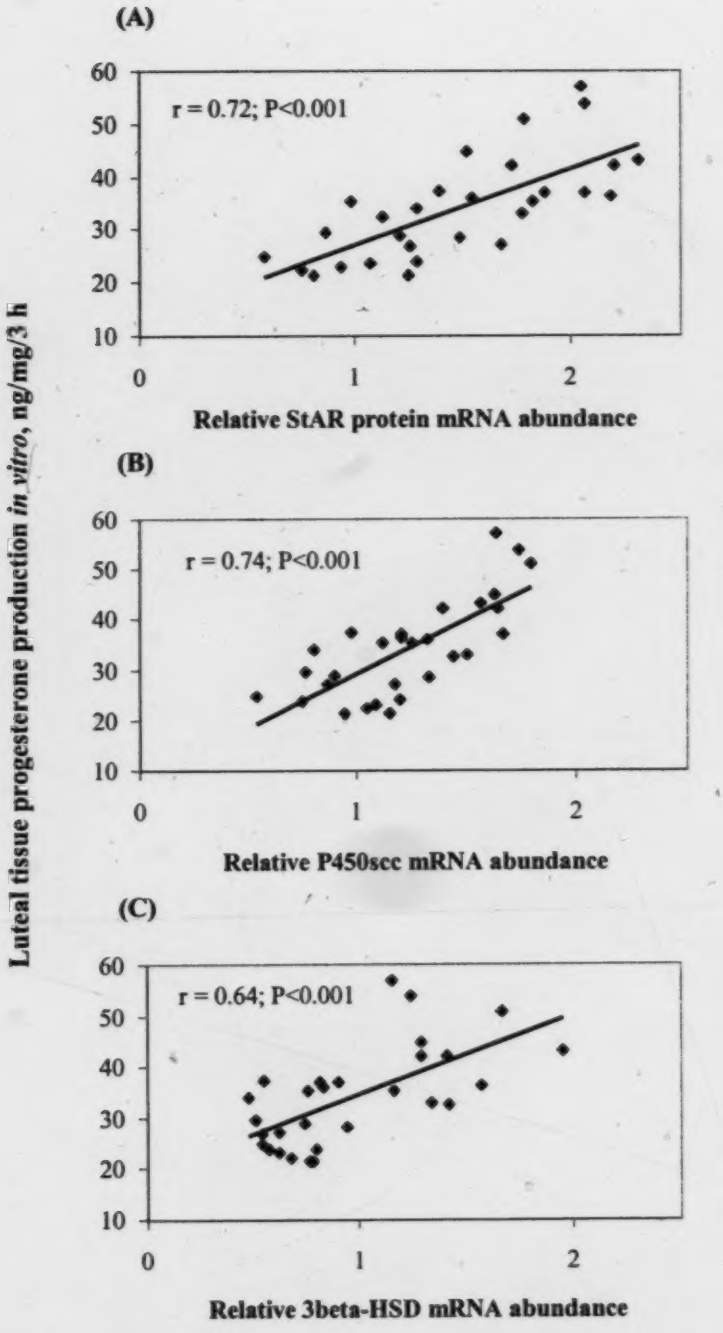
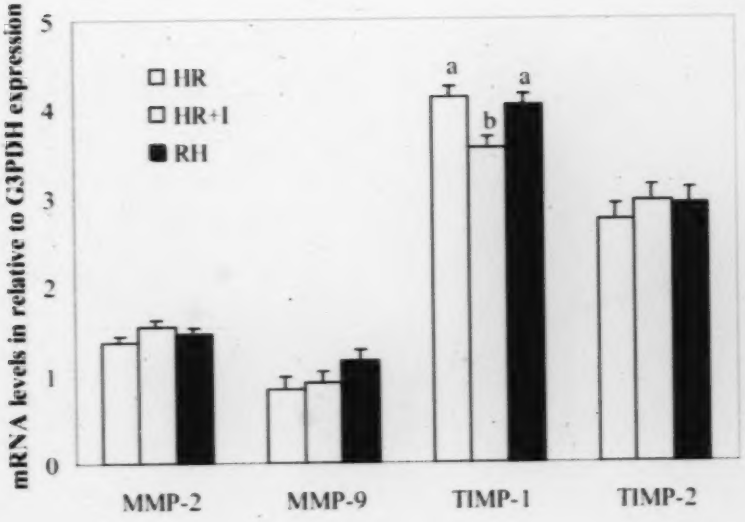
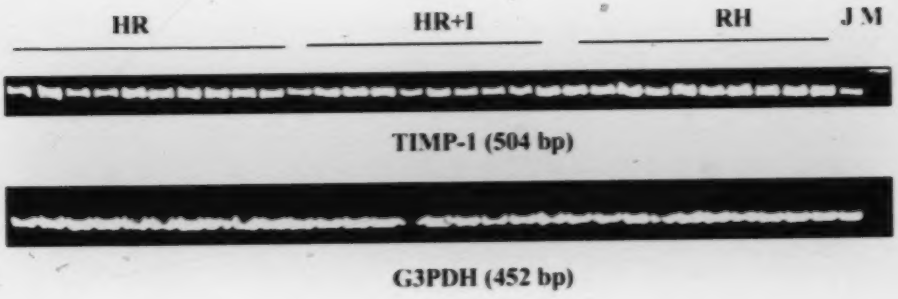

(A)
r = 0.72; P<0.001
Relative StAR protein mRNA abundance
(B)
r = 0.74; P<0.001
Relative P450scc mRNA abundance
(C)
r = 0.64; P<0.001
Relative 3beta-HSD mRNA abundance
Luteal tissue progesterone production in vitro, ng/mg/3 h
60
50
40
30
20
10
0
1
2

Figure 5.7. **A)** mRNA expression for MMP-2, MMP-9, TIMP-1, and TIMP-2 in luteal tissue from HR, HR+I and RH gilts (n=30). a, b: Means lacking common superscripts differ ($P<0.01$). Bars represent LS-means ± SEM. **B)** Representative TIMP-1 and G3PDH RT-PCR amplicons in luteal tissue from HR, HR+I, and RH gilts. Samples were organized from shortest to longest interval from left to right within treatment. J: Jag-1 cell line control. M: DNA marker.

(A)



(B) RT-PCR amplicons:



CHAPTER 6
GENERAL DISCUSSION

Nutritional requirements for growth and for milk production are very high in gilts and primiparous lactating sows, respectively (NRC, 1998). In these two groups, low feed intake during the estrous cycle and the lactation period often causes a decrease in reproductive efficiency such as low ovulation rate and low embryonic survival in the subsequent pregnancy. In our laboratory, a primiparous lactating sow model was established by Zak et al. (1997a) to study these interactions, in which differential patterns of feed intake during a 4-week lactation, and associated metabolic and endocrine changes, differentially affected the reproductive performance of the sows. Feed restriction (R) at any time during lactation (RA or AR) resulted in a lower ovulation rate and extended weaning-to-estrus intervals, compared to sows fed to appetite (A) throughout (AA). Furthermore, independent of ovulation rate, feed restriction in the last week of lactation (AR) selectively reduced embryo survival, compared to the RA sows restricted during the first three weeks of lactation. Given the importance of gilts as a component of the breeding herd, the importance of also optimizing fertility in gilts has been recognized. Moreover, cyclic gilts might represent an important experimental paradigm, as their 21-day estrous cycle can be used to represent a 21-day lactation, which is becoming the norm of the swine industry. To explore this possibility, a cyclic gilt model was also established in our laboratory, in which moderate feed restriction during the second week of the estrous cycle did not affect ovulation, but depressed progesterone concentrations in early pregnancy and decreased embryonic survival at d 28 (Almeida et al., 2000a). Thus in these experimental models, subsequent embryonic survival can be manipulated by adjusting feed intake during late lactation or the late luteal phase. These two models provide an opportunity to further investigate the mechanisms mediating nutritional effects on embryonic survival. The experiments described in this thesis were designed to investigate the mechanisms mediating nutritional effects on reproductive performance, especially on embryonic survival in the pig.

Many studies have suggested that progesterone is a mediator of nutritional effects on embryonic survival in pigs (Pharazyn et al., 1991; Hunter et al., 1996; Jindal et al., 1996 and 1997; Foxcroft, 1997; Ashworth and Pickard, 1998). An association between

progesterone concentrations in early pregnancy and embryonic survival in gilts (Pharazyn et al., 1991; Jindal, 1996) and in primiparous sows (Jindal, 1996; Zak et al., 1998; Van den Brand et al., 2000) has also been established. Data from the experiment presented in Chapter 4 confirmed that feed restriction during the last week of lactation in primiparous sows depressed plasma progesterone concentrations in early pregnancy, compared to full-fed sows.

Consistent with a role for progesterone as a mediator of nutritional effects on embryonic survival, supplementary progesterone after mating increased embryonic survival in the gilt (Ashworth, 1991; Jindal et al., 1997). However, it has been suggested that the effects of administration of progesterone on pig embryo survival are equivocal (Pope, 1994). In the first experiment reported in Chapter 3 of this thesis, we wished to further test the hypothesis that supplement of progesterone in early pregnancy in the primiparous sow would abrogate the negative effects of feed restriction, by using the same progesterone treatment protocol utilized by Jindal et al. (1997). Given the expected difference in the time interval from the onset of estrus to the preovulatory LH surge and thus ovulation between cyclic gilts and primiparous sows (Clowes et al., 1994; Jindal et al., 1997), progesterone injection was given 12 h later in primiparous sows than in gilts to make the timing of progesterone treatments comparable. Although progesterone treatment increased peripheral progesterone concentrations within 12 h, it did not improve embryonic survival.

Ashworth and Pickard (1998) indicated that only when low progesterone concentrations occur following breeding, is progesterone supplementation consistently associated with enhanced embryonic survival. The distribution of embryonic survival relative to progesterone concentrations seen in the 15 progesterone-treated sows (Figure 3.2) shows that one sow had very high survival (95%), embryo survival from seven sows fell within the range of control sows, and seven out of 15 sows had very low survival rates (<40%). At most, therefore, progesterone supplementation may only have benefited one sow, and had negative effects in as many as 7 of the sows treated. Further comparisons of plasma progesterone data in these progesterone-treated sows with data reported recently for normal sows (Van den Brand et al., 2000) indicate that exogenous progesterone treatment increased peripheral progesterone 24-36 h earlier than in normal sows. Other studies show

that progesterone concentrations from high embryo survival pigs only rise 12 h earlier than low embryo survival pigs (Hunter et al., 1996; Jindal et al., 1997; Van den Brand et al., 2000). Thus progesterone treatment in this experiment may have been initiated too soon after estrus and caused asynchrony between the development of the embryos and uterus, and was detrimental, instead of beneficial to embryonic development. Therefore, further studies on progesterone treatment protocols are needed to try and ensure that progesterone concentrations only rise 12 h earlier than the control pigs, in the hope of achieving high embryonic survival. Another conclusion might be that the mechanisms mediating nutritional effects on embryonic survival in sows are different from gilts.

Gonadotropins are essential for the late stages of follicular development and maturation (Driancourt et al., 1995). Feed restriction during the last week of lactation decreased LH secretion (Zak et al., 1997a), and affected follicle development (Quesnel et al., 1998) and oocyte maturation (Zak et al. 1997b). Exogenous GnRH treatment during lactation is very effective in stimulating ovarian follicle development (De Rensis et al., 1991) and if treatment persists a proportion of sows show estrus and ovulate during lactation (Cox and Britt, 1982; Rojanasthien et al., 1987). This led us to further test the importance of LH in mediating reproductive performance in the feed-restricted primiparous lactating sow. It was hypothesized that exogenous GnRH treatment, which restored LH secretion to that seen in unrestricted sows, would ameliorate effects of feed restriction in lactation on subsequent reproductive performance. GnRH therapy in feed restricted sows did successfully restore LH secretion to that seen in unrestricted animals. Paradoxically, however, GnRH treatment in lactation suppressed the LH and FSH responses to weaning. As a result, GnRH treatment in lactation does not appear to be effective in alleviating the metabolically induced reduction in sow fertility. In order to clearly define the immediate impact of GnRH treatment in further studies in lactating sows, it would be valuable to examine follicle development at weaning. By making sows more catabolic and depressing LH secretion, feed restriction would be expected to exaggerate suckling effects on follicle development (Zak et al., 1997b; Quesnel et al., 1998) and on embryonic survival (Zak et al., 1997a). However, making the sows anabolic during lactation by superalimenting to 125% of ad libitum feed intake did not increase LH secretion or improve reproductive performance, indicating that suckling *per se* during

lactation is the ultimate controller of fertility. The lactating sow model may therefore be a very complex model with which to study nutritional effects on fertility. Not only are the gonadotropins important for follicular development and for subsequent embryo survival, but metabolic and growth hormones acting at the ovarian level should also be taken into account.

The intraovarian growth factors (e.g. IGF-I) and metabolic hormones (e.g. insulin) play a role in regulating follicle development (Armstrong and Webb, 1997; Cosgrove and Foxcroft, 1996). These growth factors and metabolic hormones may act independently or synergistically with gonadotropins to mediate ovarian function. The role of insulin in follicle development and ovulation rate has been established in the gilt (Cox et al., 1987; Cox, 1997) and sow (Whitley et al., 1998a,b). A streptozotocin-induced diabetic gilt model was used by Meurer et al. (1991) to investigate the role of insulin in recruitment of ovulatory follicles. Insulin treatment decreased the number of atretic follicles, without any changes in gonadotropin concentrations. Similarly, in a progesterone-treated prepubertal gilt model (Cosgrove et al., 1992), 5 days of realimentation increased follicular development, independent of changes in LH secretion. Insulin treatment of feed restricted cyclic gilts counteracted the effects of feed restriction by increasing progesterone concentrations in the immediate post-ovulation period to these seen in unrestricted cyclic gilts (Almeida et al., 2000b). Other studies suggest that both insulin and IGF-I could potentiate ovarian responses to gonadotropins (Booth et al., 1996).

In the third experiment reported in Chapter 5, effects of insulin treatment on luteal function were studied using the cyclic gilt model developed by Almeida et al. (2000a). This experiment provides convincing evidence for the dynamic changes that occur in porcine luteal function in the immediate post-ovulatory period. Furthermore, feed restriction during the second week of the estrous cycle affected subsequent luteal function by decreasing the responsiveness of luteal tissue to LH stimulation *in vitro*. Concomitant insulin treatment in the feed-restricted animals (d 8 to 15) increased progesterone production and release by luteal tissue in vitro, enhanced StAR protein, P450scc, and 3beta-HSD mRNA expression, and decreased TIMP-1 mRNA expression in luteal tissue from insulin-treated gilts. Using the same population of animals, Almeida et al. (2000b) showed that feed restriction from d 8 to 15 affected peak estradiol concentrations, size of

the preovulatory LH surge, FSH concentrations in the periestrus period, and the rate of rise in plasma progesterone after ovulation. Insulin treatment counteracted all these deleterious effects and increased the rate of rise in progesterone. As previous insulin treatment had beneficial effects on luteal function and high progesterone concentrations are associated with high embryonic survival, insulin treatment may therefore improve embryonic survival in feed restricted gilts. In fact, insulin treatment in weaned sows was demonstrated to increase farrowing rate and litter size (Ramirez et al., 1997).

No differences were found in expression of mRNA for StAR protein, P450scc, 3beta-HSD in luteal tissue from feed-restricted and insulin-treated gilts. However, from a functional perspective, there was a close association between progesterone production and mRNA expression for three of the steroidogenic enzymes over the different ovulation-to-surgery time intervals. Progesterone production in vitro was also strongly correlated with circulating plasma progesterone concentrations in the gilts used for recovery of luteal tissue. These associations suggest that regulation of gene expression in differentiating luteal tissue could still be a key mechanism mediating the difference in the rate of increase in plasma progesterone reported by Almeida et al. (2000b). Our inability to demonstrate treatment-induced differences in gene expression may relate to 1) the relatively small sub-sample of gilts used for the gene expression studies; and 2) the need to impose even stricter timing on the recovery of luteal tissue to accommodate the dynamic changes in luteal function that are occurring in the immediate post-ovulatory period in pigs.

Future studies:

Based on the literature reviewed and experimental results from the lactating and weaned sows and cyclic gilts reported in this thesis, the following discussion suggests further experiments that could be used to investigate the mechanisms mediating nutritional effects on reproductive performance in pigs and further optimization of the design for such studies. Primiparous lactating sows are a principal concern. Both central and local effects of metabolic hormone and growth factor should be taken into account in lactating and weaned sows and the number of suckling piglets should also be considered. Data published in other species provide very convincing evidence to support a role of leptin, insulin and NPY in linking nutrition and reproduction. However, such data are not

available in pigs. With this information in mind, three experiments in primiparous lactating sows are proposed.

The first experiment proposed would study the importance of gonadotropin and insulin in regulating reproductive performance in lactating sows. This experiment is designed to test the hypothesis that both central (LH) and local mechanisms (insulin) are involved in regulating follicular development and maturation. Giving LH and insulin concomitantly to feed restricted lactating sows may alleviate the detrimental effects of feed restriction. Only AR lactating sows would be used and litter size would be standardized to 9-10 within 48 h after farrowing by cross fostering. Sows would then be allocated one of three treatments. AR sows, fed to appetite from farrowing until d 21 and restricted to 50% of ad libitum feed intake from d 22 to 28 as described by Zak et al. (1997a); AR+LH sows, fed as AR sows but receiving 800 ng of GnRH every 6-h based on the data of De Rensis et al. (1991) and the results of the work reported in Chapter 4 of this thesis; AR+LH+Insulin sows, fed as AR sows, given LH every 6 h and long-acting insulin *s.c.* twice daily as described in the studies of Almeida et al. (2000b). On both d 21 and 28, blood samples would be taken every 15 min for a 12-h period to characterize LH, and hourly for determining insulin, leptin and IGF-I concentrations.

In order to avoid the problem that GnRH treatment during lactation in feed-restricted sows produces inhibitory effects on LH secretion in response to weaning, reported in Chapter 4, all the animals in this experiment would be slaughtered at weaning and both ovaries collected for analysis. Immediately after the animals are terminated, hypothalamic tissue would be collected and snap frozen in liquid nitrogen for Northern Blotting analysis of NPY mRNA expression and hypothalamic NPY content. The number of large (>5 mm in diameter), medium (3-5 mm) and small (<3 mm) size follicles on both ovaries would be recorded. Based on the approach of Quesnel et al. (1998), follicle fluid harvested from the largest 10 follicles would be used for determining IGF-I, IGFBP, estradiol and inhibin concentrations.

As discussed above, progesterone may be an important mediator of embryonic survival in pigs. However, it is not clear if feed restriction during lactation will have latent effects on luteal function as seen in cyclic gilts. This data will also help to decide if progesterone therapy should be used in the sow or not. Therefore, a second experiment is

suggested to determine progesterone production *in vitro* and mRNA expression in luteal tissue in primiparous weaned sows, using the same approach described in Chapter 5. After farrowing, litter size would be adjusted to 9-10 for each sow by cross fostering and sows divided into two treatment groups. AA sows would be fed to appetite throughout 28-d lactation period and AR sows fed as AA sows during the first three week of lactation and then feed restricted from d 22 to 28. All sows would be weaned at d 28 and checked for the onset of estrous. Thirty-six h after the onset of estrus, ultrasound would be performed to monitor the time of ovulation. Based on the results reported in Chapter 4, in which progesterone concentrations were different between AA and AR sows at 96 h (≈ 42 h after ovulation) after onset of estrus, all sows would be slaughtered at 42 h after ovulation to collect ovarian tissue for in vitro luteal tissue culture. Enough luteal tissue would be harvested and snap frozen in liquid nitrogen for the analysis of expression of mRNAs coding for steroidogenic enzymes. After estrus, a series of blood samples would be taken to characterize the rise in progesterone. This experiment would provide a good understanding of the effects of feed restriction during later lactation on subsequent luteal function. The plasma progesterone data would provide a better idea about the precise time difference in the progesterone rise between the two groups. Therefore, the progesterone therapy approach may be taken again to improve embryonic survival in the primiparous sows, if appropriate.

The third experiment proposed aims to verify the lactating sow model and investigate the effects of number of piglets suckling on LH secretion and follicular development in GnRH treated AR sows. Suckling by piglets is the ultimate inhibitor of LH secretion during lactation (Zak et al., 1998) and this effect confounds the effects of feed restriction. Compared to AA sows suckling 6 piglet (Zak et al., 1997a), the embryonic survival from the lactating and weaned sows that suckled 9 to 10 piglets, reported in Chapter 4, was lower (67 vs. 87.5%). To get a better understanding of the importance of litter size in inhibiting LH secretion and effects on follicular development, this experiment is designed to investigate the effects of suckling by 6 or 12 piglets on the outcome of GnRH treatment. Again, the AR sow model would be used and litter size would be standardized to 6 (AR-6) or 12 (AR-12) within 48 h after farrowing. Therefore, the metabolic states between AR-6 and AR-12, would be different (Zak et al. 1997a; Mao

and Foxcroft, 1998). Therefore, to avoid the confounding effects of differences in metabolic state between the two groups, feed intake would be adjusted to achieve similar energy balance based on the sow body weight, number of piglets and litter gain (Noblet et al., 1990). Feed intake in all sows from d 22 to weaning would be restricted to 50% of the average feed intake from d 17 to 21. GnRH injections would be given every 6 h during the last week of lactation. At weaning on d 28, sows would be killed. Both ovaries and hypothalamic tissue would be treated the same way as in the first experiment proposed. Any differences found in this experiment would help to design an even better lactating sow model, in which confounding effects may be controlled by reducing the number of suckling piglets.

Insulin treatment in cyclic gilts proved to be beneficial for luteal function by increasing progesterone production and enhancing gene expression for key steroidogenic enzymes reported in Chapter 5. Insulin treatment also increased the rate of rise in plasma progesterone after ovulation (Almeida et al., 2000b). However, whether these effects will have any beneficial effects on embryonic survival and the final outcome of litter size is not known. Therefore, the last experiment is proposed to be carried out in cyclic gilts to determine the effects of insulin treatment on embryonic survival and litter size. This experiment would extend the observations reported in Chapter 5. As described by Almeida et al. (2000b), HR and HR+I gilt models would be used in this experiment and fed to 2.8 × maintenance requirements from d 1 to 7 (day 0 = estrus). From d 8 to 15, all gilts would be fed to 2.1 × maintenance and treated with (HR+I), or without (HR) insulin twice daily. Then gilts would be fed 2.8 × maintenance until estrus. Gilts would be checked for the onset of estrus every 12 h from expected d 18 of the cycle and bred twice at 12 and 24 h after onset of estrus. After mating, all gilts would be fed according to the NRC recommendation (1998) for pregnant sows. Half the animals from each group would be killed to determine ovulation rate and embryonic survival at d 28 of pregnancy. The other half of the animals would go through the gestation and farrow. At farrowing, litter size would be recorded.

The results of this proposed series of experiments would provide a better understanding of nutritional effects on reproductive performance in sows and suggest a reliable way to alleviate nutritional effects on embryonic survival.

LITERATURE CITED

Almeida FRCL, Kirkwood RN, Aherne FX, Foxcroft GR. Consequences of different patterns of feed intake during the estrous cycle in gilts on subsequent fertility. J Anim Sci 2000a; 78: 1556-1563.

Almeida FRCL, Mao J, Novak S, Cosgrove JR, Foxcroft GR. Effects of patterns of feed restriction and insulin treatment during the luteal phase on reproductive, metabolic and endocrine parameters in cyclic gilts. 2000b; (submitted to J Anim Sci).

Armstrong DG, Webb R. Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. Rev Reprod 1997; 2: 139-146.

Ashworth CJ. Effect of pre-mating nutritional status and post-mating progesterone supplementation on embryo survival and conceptus growth in gilts. Anim Reprod Sci 1991; 26: 311-321.

Ashworth CJ, Pickard AR. Embryo survival and prolificacy. In: Progress in Pig Science. Nottingham, UK: Nottingham University Press. 1998; 303-325

Booth PJ, Cosgrove J, Foxcroft GR. Endocrine and metabolic responses to realimentation in feed-restricted prepubertal gilt: Associations among gonadotrophins, metabolic hormones, glucose, and utero-ovarian development. J Anim Sci 1996; 74: 840-848.

Clowes EJ, Aherne FX, Foxcroft GR. Effect of delayed breeding on the endocrinology and fecundity of sows. J Anim Sci 1994; 72: 283-291.

Cosgrove JR, Foxcroft GR. Nutrition and reproduction in the pig: ovarian aetiology. Anim Reprod Sci 1996; 42: 131-141.

Cosgrove JR, Tilton JE, Hunter MG, Foxcroft GR. Gonadotropin-independent mechanisms participate in ovarian responses to realimentation in feed-restricted prepubertal gilts. Biol Reprod 1992; 47: 736-745.

Cox NM. Control of follicular development and ovulation rate in pigs. J Reprod Fertil Suppl 1997; 52: 31-46.

Cox NM, Britt JH. Pulsatile administration of gonadotropin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus. Biol Reprod 1982; 27: 1126-1137.

Cox NM, Stuart MJ, Althen TG, Bennett WA, Miller HW. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J Anim Sci 1987; 64: 507-516.

De Rensis F, Hunter MG, Grant SA, Lancaster RT, Foxcroft GR. Effect of estrogen administration on endogenous and luteinizing hormone-releasing hormone-inducing

luteinizing hormone secretion and follicular development in the lactating sow. Biol Reprod 1991; 44: 975-982.

Driancourt MA, Locatelli A, Prunier A. Effects of gonadotrophin deprivation on follicular growth in gilts. Reprod Nutr Dev 1995; 35: 663-673.

Foxcroft GR. Mechanisms mediating nutritional effects on embryonic survival in pigs. J Reprod Fertil Suppl 1997; 52: 47-61.

Hunter MG, Picton HM, Biggs C, Mann GE, McNeilly AS, Foxcroft GR. Periovulatory endocrinology in high ovulating Meishan sows. J Endocrinol 1996; 150: 141-147.

Jindal, R. Physiological mechanisms mediating nutritional effects on embryonic survival in the pig. Edmonton, CA: University of Alberta; 1996. Ph.D. Thesis.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. J Anim Sci 1996; 74: 620-624.

Jindal R, Cosgrove JR, Foxcroft GR. Progesterone mediates nutritionally induced effects on embryonic survival in gilts. J Anim Sci 1997; 75: 1063-1070.

Mao J, Foxcroft GR. Progesterone therapy during early pregnancy and embryonal survival in primiparous weaned sows. J Anim Sci 1998; 76: 1922-1928.

Meurer KA, Cox NM, Matamoros IA, Tubbs RC. Decreased follicular steroids and insulin-like growth factor-I and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J Reprod Fertil 1991; 91: 187-196.

Noblet J, Dourmad JY, Etienne M. Energy utilization in pregnant and lactating sows: Modeling of energy requirements. J Anim Sci 1990; 68: 562-572.

NRC. Nutrient Requirements of Swine (10^{th} Revised Ed.). National Academy Press, Washington, DC. 1998.

Pharazyn A, den Hartog LA, Foxcroft GR, Aherne FX. Dietary energy and protein intake, plasma progesterone and embryo survival in early pregnancy in the gilt. Can J Anim Sci 1991; 71: 949-952.

Pope WF. Embryonic Mortality in Swine. In: Zavy MT, Geisert RD (eds.), Embryonic Mortality in Domestic Species. CRC Press. 1994; 53-77

Quesnel H, Pasquier A, Mounier AM, Prunier A. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J Anim Sci 1998; 76: 856-863.

Ramirez JL, NM Cox, Moore AB. Influence of exogenous insulin before breeding on conception rate and litter size of sows. J Anim Sci 1997; 75: 1893-1898.

Rojanasthien SAM, Lundeheim N, Einarsson S Luteinizing hormone response to different doses of synthetic gonadotrophin releasing hormone during early and late lactation in primiparous sows. Anim Reprod Sci 1987; 13: 299-307.

Van den Brand H, Soede NM, Kemp B. Dietary energy source at two feeding levels during lactation of primiparous sows: II. Effects on periestrus hormone profiles and embryonal survival. J Anim Sci 2000; 78: 405-411.

Whitley NC, Moore AB, Cox NM. Comparative effects of insulin and porcine somatotropin on postweaning follicular development in primiparous sows. J Anim Sci 1998a; 76: 1455-1462.

Whitley NC, Thomas MNQ, Skelton JO, Moore AB, Purvis J, Qiu Y, Cox NM. Influence of insulin on follicular development and the intrafollicular insulin-like growth factor I (IGF-I) system in sows after weaning. J Reprod Fertil 1998b; 112: 175-184.

Zak LJ, Cosgrove JR, Aherne FX, Foxcroft GR. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J Anim Sci 1997a; 75: 208-216.

Zak LJ, Xu X, Hardin RT, Foxcroft GR. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J Reprod Fertil 1997b; 110: 99-106.

Zak LJ, Williams IH, Foxcroft GR, Pluske JR, Cegielski AC, Clowes EJ, Aherne FX. Feeding lactating primiparous sows to establish three divergent metabolic states: I. Associated endocrine changes and postweaning reproductive performance. J Anim Sci 1998; 76: 1145-1153.